PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
C07K 15/06, A61K 39/395, C07K 15/28

A1

(11) International Publication Number: WO 94/25489

(43) International Publication Date: 10 November 1994 (10.11.94)

(21) International Application Number: PCT/CA94/00228

(22) International Filing Date: 26 April 1994 (26.04.94)

(30) Priority Data:
9308581.9 26 April 1993 (26.04.93) GB

(71) Applicant *(for all designated States except US)*: UNIVERSITY OF MANITOBA [CA/CA]; Winnipeg, Manitoba R3T 2N2 (CA).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: MOHAPATRA, Shyam, S. [CA/CA]; 364 Lindenwood Drive East, Winnipeg, Manitoba R3P 2H1 (CA). SEHON, Alec, H. [CA/CA]; 695 Academy Road, Winnipeg, Manitoba R3N 0E8 (CA).

(74) Agent: STEWART, Michael, I.; Sim & McBurney, Suite 701, 330 University Avenue, Toronto, Ontario M5G 1R7 (CA).

(81) Designated States: AU, BR, CA, CN, FI, JP, KR, NO, NZ, RU, UA, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: DOWNREGULATION OF ALLERGEN-SPECIFIC IMMUNE RESPONSE

(57) Abstract

Synthetic peptides are provided for effecting immunotherapy. The peptides have an amino acid sequence comprising at least a portion of the CDR3 region of a T-cell receptor and capable of eliciting a T-cell response, particularly an amino acid sequence encoded by a nucleotide sequence comprising the J-gene of the α-chain and/or β-chain of the TCR. The peptides are particularly useful in suppressing an immune response to an antigen, particularly an allergenic antigen.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**GB**	United Kingdom	**MR**	Mauritania
AU	Australia	**GE**	Georgia	**MW**	Malawi
BB	Barbados	**GN**	Guinea	**NE**	Niger
BE	Belgium	**GR**	Greece	**NL**	Netherlands
BF	Burkina Faso	**HU**	Hungary	**NO**	Norway
BG	Bulgaria	**IE**	Ireland	**NZ**	New Zealand
BJ	Benin	**IT**	Italy	**PL**	Poland
BR	Brazil	**JP**	Japan	**PT**	Portugal
BY	Belarus	**KE**	Kenya	**RO**	Romania
CA	Canada	**KG**	Kyrgystan	**RU**	Russian Federation
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SD**	Sudan
CG	Congo			**SE**	Sweden
CH	Switzerland	**KR**	Republic of Korea	**SI**	Slovenia
CI	Côte d'Ivoire	**KZ**	Kazakhstan	**SK**	Slovakia
CM	Cameroon	**LI**	Liechtenstein	**SN**	Senegal
CN	China	**LK**	Sri Lanka	**TD**	Chad
CS	Czechoslovakia	**LU**	Luxembourg	**TG**	Togo
CZ	Czech Republic	**LV**	Latvia	**TJ**	Tajikistan
DE	Germany	**MC**	Monaco	**TT**	Trinidad and Tobago
DK	Denmark	**MD**	Republic of Moldova	**UA**	Ukraine
ES	Spain	**MG**	Madagascar	**US**	United States of America
FI	Finland	**ML**	Mali	**UZ**	Uzbekistan
FR	France	**MN**	Mongolia	**VN**	Viet Nam
GA	Gabon				

TITLE OF INVENTION

DOWNREGULATION OF ALLERGEN-SPECIFIC IMMUNE RESPONSE

FIELD OF INVENTION

The present invention relates to the discovery that the antibody (Ab) production in a mammal can be specifically suppressed to a given allergenic antigen (Ag) by treatment with a peptide(s) corresponding to the sequence of the junctional segment of the α and/or β chain of the Ag receptor of suppressor T (Ts) cells, which have been induced by the tolerogenic conjugates of the antigen in question with monomethoxypolyethylene glycol (mPEG). This method is useful for the treatment of a broad spectrum of immunological diseases, including diseases due to an aberration of the immunological system, as is the case in allergic and asthmatic disorders.

BACKGROUND TO THE INVENTION

A) Immunotherapy of allergic diseases:

IgE-mediated allergic diseases, such as hayfever and asthma, are induced by a number of antigens present in a variety of environmental antigens such as the multideterminant proteins or glycoproteins in pollens in ~20% of the genetically predisposed population (ref. 1 - a list of the references appears at the end of the specification). In contrast to some other allergens (ref. 2) (e.g., cat or house dust mite allergens), the global distribution of pollens of a large variety of grasses, trees and weeds preclude any realistic possibilities that individuals allergic to pollen allergens can avoid these aero-allergens. The current main treatment for hayfever consists primarily in symptomatic relief. Sufferers take drugs, such as anti-histamines and steroids, which do not suppress the formation of IgE antibodies and often have harmful side effects.

Attempts to downregulate the IgE immune responses of allergic subjects by the "time-honoured" immunotherapy consist of a series of injections of increasing amounts of the allergenic extracts of the appropriate pollen or pollen-mixtures over prolonged periods lasting usually 3 to 5 years. Most of the pollen extracts used therapeutically are crude mixtures of a multiplicity of chemical constituents. Some of these components bear no relation whatsoever to the few allergenic components which are actually responsible for a given patient's hypersensitivity. Because some of the proteins present in these extracts may not be allergens, standardization of allergenic extracts based on total protein content is an unreliable guide for determining the potency of an extract. Moreover, large (up to 100x) variations in allergen content occur in the preparations used for immunotherapy because of (i) the different methods used for pollen collection and storage, which lead to variations in raw materials from lot to lot and from year to year, and (ii) the crudity of the extraction procedures. Therefore, although different patients may be allergic to different consitutents of a given pollen, all patients receive injections of the "same" complex mixture containing all the constituents of different pollens, i.e., they receive even components to which they may not be allergic. It is, therefore, not surprising that treatment with an ill-defined pollen extract may lead to the induction of additional IgE antibodies, i.e., to sensitization of the patients to new components (refs. 3 to 6).

While up to 80% of patients gain clinical improvement from this therapy (refs. 7, 8), the risk of side effects, the lengthy course of therapy, the inconvenience to the patient of the mode and frequency of administration, and the mounting costs of this treatment limit the utility of current immunotherapy. Although

local and systemic reactions may occur as a result of this therapy, they may be managed by a physician specialized in allergy. However, occasionally this mode of treatment is associated with the risk of severe asthmatic or anaphylactic reactions, which can result in death (refs. 9, 10). Several laboratories have isolated some of the allergens from the crude aqueous extracts of grass pollens by the use of classical physicochemical methods and reverse immunosorbents consisting of immobilized murine monoclonal antibodies to the pollen constituents (refs. 11, 12). The main drawback of these extremely labour intensive purification methods is the minute yield of allergens. Moreover, these methods do not ensure absolute purity of the allergenic constituents and, therefore, the determination of their amino acid sequences is difficult, if not impossible. As a corollary, the development of new therapeutic derivatives of grass pollen allergens for pollen allergies are severely restricted by the use of allergens isolated by the existing procedures. Recent innovations in recombinant DNA (rDNA) technology have paved the way for the synthesis, on an industrial scale and in a consistently pure state of allergenic proteins and of their epitopic fragments responsible for their activation of the appropriate B and T cells leading interactively to IgE formation. To this end a number of allergens have been cloned (refs. 13 to 18). However, in view of the multiplicity of allergens and of their epitopes, the task of selecting allergens for therapy remains formidable and to-date recombinant allergens have not been utilized in controlled studies for the evaluation of their safety and clinical efficacy.

B) Immunopathology and immune modulation of allergic response:

Upon initial exposure, allergens present in the extracellular fluids of the body (as all other antigens)

are internalized by antigen-presenting cells (APC), which include nonantigen-specific phagocytic cells or specific B cells, and "processed" by these cells. The net effect of this processing is the breakdown of the antigens into peptidic determinants which, in turn, are re-expressed in association with class I or class II molecules of the major histocompatibility complex (MHC) on the surface of the APC. Subsequently, the binary peptide-MHC complexes interact with the corresponding specific T-cell receptors (TCR) of, respectively, (i) helper T (Th) cells or (ii) CTLs or suppresser T (Ts) cells, and the resulting triads determine the up- or down-regulation of the appropriate B cells (ref. 19).

On the basis of their lymphokine secretion patterns, the Th cell subpopulation may be further subdivided into three subsets, i.e., Th0, Th1 and Th2 cells (ref. 20). In mice and man, the Th2 cells have been shown to produce IL-4, IL-5 and IL-6, and IL-4 has been shown to activate B cells leading to the production of IgE antibodies. By contrast, the Th1 cells produce IFNγ which blocks the production of IL-4. The mechanism(s) involved in the suppression of antibody responses by Ts cells is still not fully understood. It has been suggested that the suppression of antibody production is due to inactivation of a specific Th cells as a result of non-professional APC, i. e., a T cell (ref. 21).

The primary reaction of the IgE antibodies secreted from the Bε cells is their binding to specific IgE receptors on the surface of mast cells, basophils and eosinophils. On re-exposure of the patient to the specific multivalent allergen, the cell-fixed IgE antibodies react with and are crosslinked by the allergenic molecules, which leads to the release from these cells of chemical mediators of anaphylaxis. In turn, these mediators act rapidly on the smooth muscles of different target organs resulting in the inflammatory

manifestations characteristic of the hypersensitivity diseases of the immediate type. It is obvious, from this simplistic overview of the complex cellular interactions leading to IgE production and to its effector mechanisms, that the most effective therapy ought to be directed toward the downregulation, if not total abrogation, of IgE production which is the culprit isotype responsible for immediate hypersensitivity manifestations. It has been considered that the development of therapeutic strategies (ref.22) that could influence the formation of IgE antibodies requires detailed knowledge of (i) the structures of individual allergens and, in particular, of their B cell epitopes (which are recognized by IgE/IgG antibodies), (ii) structures recognized by MHC molecule (i.e., Ta epitope), and (iii) the structures recognized by T cell receptors of Th or Ts cells.

C) Molecular biology of T cell tolerance:

Induction of tolerance to T cell dependent antibody immune responses is considered to be of pivotal importance in devising appropriate therapeutic interventions for several immunologic diseases. Several mechanisms, involving clonal deletion, clonal anergy, and suppressor T (Ts) cells have been postulated to be involved in the induction of immunological tolerance (ref. 23). During the last decade, advances in recombinant DNA and cell manipulation technologies, which are used for establishment of transgenic and mutant mice, have led to the elucidation of some of these mechanisms (ref. 24). The majority of these studies have focused on T cell antigen receptors (TCRs) which play a key role in initiating or down-regulating an immune response on their interaction with the appropriate antigenic epitope presented as a complex with MHC molecules of the antigen presenting cell.

The TCRs of helper (Th), cytotoxic (Tc) and Ts cells consist of cell surface heterodimers of $\alpha\beta$ or $\gamma\delta$ chains

(refs. 25 to 30). Each of these chains is composed of regions encoded by separate gene segments: variable (V), junctional (J) and constant (C) elements, in addition the β chain contains a diversity (D) segment (ref. 31). These gene elements are organized and rearranged in a fashion similar to those of immunoglobulins. The functional repertoire of TCRs is restricted because of the limited usage of TCR chains detected on T cells when primed with antigens (Ags) (ref. 32). Thus, a few Vα and/or Vβ genes appear to be selected for specific T cell responses as exemplified by different antigenic systems, such as cytochrome C (ref. 33), p-azobenzene arsonate (ref. 34), λ repressor fragment 12-20 (ref. 35), myelin basic protein (MBP) (ref. 36) and sperm whale myoglobin (ref. 37). Moreover, as a result of the findings that (i) TCRs recognize the antigenic peptides in the context of a complex with MHC molecules of APCs and (ii) some antigenic peptides are associated with either the α or β chain of the TCR, it was postulated that whereas one of these chains may be involved in Ag binding, the other is involved in binding to the appropriate MHC molecule (ref. 38). In a variation of this theme, it was proposed that whereas the process of T cell recognition involves the binding of the complementarity determining region 3 (CDR3) to the peptide in question, the CDR1 and CDR2 regions contact the α helices of the MHC molecule (ref. 26). Recent studies have also established that the size of the peptides bound to MHC class I and class II molecules are limited, respectively, to 9 to 12 and 13 to 25 amino acid residues (ref. 39), and that the interaction between the peptide and the TCR might involve $\geq$4 residues of the V-J regions of α and/or β chain(s) (ref. 40).

A number of studies employing transgenic mice carrying TCR β transgenes have revealed that the rearrangement and expression of TCR β genes play a

paramount role in shaping the T cell repertoire in the thymus (ref. 41). Thus, thymocytes are subjected to both positive and negative selection, which results in the development of single positive functional thymocytes. Furthermore, recent studies utilizing the mutant (knock-out) mice, which lacked TCR β or TCR α chains, led to the conclusion that a singly rearranged β chain was sufficient to drive the transition from double negative to double positive thymocytes, whereas the TCR α chain mediated the transition from double positive to single positive thymocytes (ref. 42). Thus, whereas the mechanisms of positive and negative selection, which are operative in the thymus and which ensure self-tolerance, are becoming somewhat clearer, the mechanisms for induction of tolerance or downregulation of specific aberrant responses in extra thymic organs and in the periphery are poorly understood. Nevertheless, it is to be noted that employing transgenic mice for TCR α and β receptors, it has been shown that clonal anergy accounts often for the tolerance in the periphery (ref. 43).

On the basis of the observations that the TCR V gene usage in immune responses to certain auto-Ags (e.g., Myelin basic protein (MBP) involved in EAE and collagen involved in arthritis), was restricted to a few genes (ref. 44), it has been suggested that TCR peptides may serve as reagents for immunoregulation in autoimmune diseases (refs. 45 to 47). Thus, mice susceptible to MBP were found to utilize mainly the Vβ 8.2 gene (ref. 44) and their pretreatment with the Vβ 8.2 peptide 39-59, emulsified in adjuvant, induced cross-reactive and EAE protective T cells and Abs (refs. 45, 46). Moreover, it has been reported that treatment with the TCR peptide even after the induction of EAE led to a significant amelioration of the condition. In other auto-immune conditions, involving more than 3-4 different TCR V genes, a mixture of peptides led to prevention of the

progress of the disease (ref. 47). In essence, it may be inferred from these studies that (i) certain TCRs, which may be either released by T cells in soluble form or shed from these cells, may induce regulatory networks, and (ii) these networks may be effectively utilized to downregulate the immune responses to specific pathogenic Ags in the periphery.

In relation to allergic diseases, recently studies have implicated the pivotal role of TCR genes in the allergic immune response. For instance, the antigen receptors utilized in experimental Leishmaniasis (ref. 48), and OVA-specific IgE responses in mice (ref. 49) were restricted. Moreover, analysis of T cell receptor genes of human T cell clones specific to dustmite allergens (ref. 50) and to those of grass pollen allergens (ref. 51) revealed restricted usage of TCR genes.

D) Induction of specific tolerance by Ag-mPEG conjugates:

A number of strategies may be utilized for induction of specific tolerance, which has been reviewed recently (ref. 52). One of the methods included administration of the tolerogenic antigen-mPEG conjugates to the animal prior to the injection of antigen (refs. 53 to 55). In previous studies, it was shown that covalent grafting of an optimal number(n) of polyethylene glycol or of mPEG molecules onto various protein Ags and allergens resulted in the loss of most of their antigenicity and allergenicity (refs. 56, 57). Furthermore, the administration of Ag(mPEG)n conjugates into rats and mice, prior to or at the time of immunization with the unmodified Ag resulted in abrogation of Ag-specific IgE and IgG Ab responses (refs. 53 to 57). Similarly, it was shown in extensive studies with mPEG conjugates of human IgG (HIgG) that administration of these conjugates, prior to immunization with heat-aggregated HIgG (haHIgG), resulted in specific tolerance to HIgG as manifested by

a marked (>90%) reduction in the level of anti-HIgG Abs on subsequent multiple injections of haHIgG, at intervals of about 4 weeks, even over a period of >300 days (refs. 58, 59). Moreover, it was shown that the specific tolerance induced in mice by the Ag(mPEG)$_n$ conjugates was transferable to normal mice by unfractionated spleen cells, or by sIg^-, $CD8^+$ splenic cells (ref. 59), or their crude freeze-thaw extract (FTE), or by a suppressor factor (TsF) released from these cells by their interaction with an immunosorbent consisting of a moAb to TCR α chain of T cells. As in the donors of the Ts cells, the tolerance induced in recipients of either Ts cells or their FTE or TsF lasted for extended periods (at least 90 days) (ref. 60).

The downregulation of Ag-specific Ab responses by the Ag-mPEG conjugates was investigated by deriving Ts cell clones specific to HIgG (e.g., clone 23.32) or specific to OVA (e.g., clone 17.2), which were generated from the spleen cells of mice which had been tolerized by treatment with HIgG(mPEG) or OVA(mPEG), respectively (refs. 61 to 63). The addition of the cloned T cells or of their FTE led to a dose-dependent, MHC-restricted downregulation of the anti-DNP Ab response. Furthermore, the 23.32 FTE suppressed the anti-DNP Ab responses by the downregulation of HIgG-specific Th cells (ref 62). Moreover, the analysis of the cytokine production by 3 OVA-specific Ts cell clones by the use of bioassays, and by Western and Northern blot, demonstrated that upon activation with anti-CD3 mAbs, the cloned Ts cells produced IL2, TNFα, TGFβ, TNFβ, IFNγ and IL4. However, the addition of anti-TNFα, anti-TNFβ, anti-IFNγ or anti-IL-4 mAbs, to the <u>in vitro</u> Ab forming system did not abrogate the suppression of Ab production by these Ts cells. Moreover, addition of anti-TGFβ mAb did not abrogate the tolerance induced by FTE of these cells. Thus, it may be concluded that the cytokine profile of

the cloned $CD8^+$ T cells is similar to, but not identical with that of CTL (ref. 63), and the observed suppression mediated by these Ts cells is not exclusively due to production of TNFα, TNFβ, IFNγ and TGFβ.

By the use of appropriate monoclonal antibodies (mAbs) to T cell markers in conjunction with cytofluorimetric analysis, the cloned Ts cells were shown to be $Thy\text{-}1^+$, $CD4^-$, $CD5^-$ and $CD8^+$, and >95% of the cloned cells co-expressed CD3 and the $\alpha\beta$ TCR. By the use of immunosorbents, incorporating mAbs to the α and β chain epitopes of TCR, it was demonstrated that whereas the FTE of clones 23.32 shared the epitopes of the α chain of TCR (ref. 61), the FTE of cells of clone 17.2 was serologically and structurally related to the α/β heterodimer of TCR. Furthermore, Western blotting of the partially purified FTE of the T cells 17.2 by affinity chromatography with either of the two immunosorbents, incorporating the moAbs to α or β chain, revealed that the 17.2 TsF was a two chain disulfide-linked molecule with a MW of 84K, and consisted of 2 subunits of 42 KD each (ref. 64). Moreover, the two-chain heterodimer, isolated by SDS polyacrylamide gel electrophoresis, had the capacity to downregulate the <u>in vitro</u> Ab production in an Ag-specific manner. These studies suggested that a soluble form of the α and or β chain of TCR may play a cardinal role in the Ag-specific downregulation of Ab responses.

SUMMARY OF INVENTION

This invention involves the development of a method for the induction of tolerance to specific antigens by treatment of a mammal with a peptide segment of the T-cell receptor (TCR) α and/or β chain(s) of Ts cells, which cells were induced <u>in vivo</u> by tolerogenic Ag-mPEG conjugates.

Accordingly, in one aspect, the present invention provides a synthetic peptide having an amino acid

sequence comprising at least a portion of the complementarity determining region 3 (CDR3) region of a T-cell receptor, particularly a human T-cell receptor, of an antigen, particularly an allergen, and which is capable of eliciting a T-cell response.

In one embodiment of this aspect of the invention, the amino acid sequence is encoded by a nucleotide sequence comprising the J-gene of the α-chain of the TCR, or by a nucleotide sequence comprising the J-gene of the β-chain of the TCR. A mixture of such peptides is particularly useful in immunotherapy, as described in more detail below, one peptide being encoded by a nucleotide sequence comprising the J-gene of the α-chain of TCR and another encoded by a nucleotide sequence comprising the J-gene of the β-chain of the TCR.

The synthetic peptides provided herein may have about 8 to about 12 amino acids and be capable of binding to a class I MHC molecule or about 12 to about 27 amino acids and be capable of binding to a class II MHC molecule.

The peptides of the invention are useful in immunotherapy. Accordingly, in another aspect, the present invention provides a method of immunotherapy, which comprises administering to a host, particularly a human, a peptide according to the invention or an immunosuppressive composition comprising at least one such peptide and a pharmaceutically-acceptable carrier. Such composition may further comprise an adjuvant.

The host may have previously exposed to the antigen or the material may be administered for prophylatic immunization of the host. The immunization may be effected to suppress an allergic response in the host to the antigen or to protect the host from an allergic response to the antigen. The immunization also may be effected to suppress an autoimmune response in the host to the antigen or to protect the host from an autoimmune

response to the antigen. The immunotherapeutic treatment may be effected herein in conjunction with the administration of therapeutic molecules prone to producing unwanted immunological responses.

The peptides of the present invention also are useful in screening a host for an immunogenic response to an antigen, particularly an allergen. Accordingly, in a further aspect of the invention, there is provided a method of diagnosing an allergic response of a host to an allergen, which comprises screening a serum from the host with a plurality of the peptides according to the invention and corresponding to a plurality of allergenic antigens, and detecting reactivity of at least one of the peptides to the serum as a detection of an allergen to which the host has been exposed. The at least one peptide which is detected in this manner then may be administered to the host as an immunotherapeutic treatment according to the method described above.

The present invention also includes a method of identifying the amino acid sequence of the peptides provided herein. Accordingly, in an additional aspect of the present invention, there is provided a method of identifying a peptide having an amino acid sequence comprising at least a portion of the CDR3 region of a T-cell receptor of an antigen and capable of eliciting a T-cell response, which comprises effecting induction of regulatory T-cells to a desired antigen, particularly an allergen, determining the nucleotide sequence of T-cell receptors of the regulatory T-cells, determining the portion of the nucleotide sequence of the T-cell receptors which codes for the CDR3 region of the T-cell receptors, and deducing the amino acid sequence of the determined portion of the nucleotide sequence as a determination of the amino acid sequence of the peptide.

The regulatory T-cells preferably are induced by administration to a host of a conjugate of a non-

immunogenic substrate, particularly a polymer, and the desired antigen. The polymeric substrate may be selected from carboxymethyl celluloses, monomethoxypolyethylene glycols (MPEGs) and polyvinyl alcohols. Preferably, the conjugate comprises an antigen - MPEG conjugate.

The regulatory T-cells induced by the antigen-substrate conjugates generally comprises $CD8^+$ cells and the nucleotide sequence of T-cell receptors of such cells are determined, generally by conventional cloning procedures.

The determination of the CDR3 region of the T-cell receptors preferably comprises determining the individual nucleotide sequences for the α- and β-chains of the T-cell receptor and effecting sequence analysis of the individual sequences to determine the sequences of the J-gene of both α- and β-chains.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 contains a schematic representation of anchored PCR protocols for amplification of 5′ end of the α chain of Ts clones. Total RNA was reverse-transcribed with a gene-specific primer, $C\alpha E_3$, to create a (-) strand of cDNA (hatched box). A poly-(A) tail was added, annealed with dT_{17} adaptor primer and extended to generate the (+) strand. Then, a gene-specific internal oligo primer, Cα5RT, and the adaptor-primers were used for further amplification.

Figure 2 contains the nucleotide sequence (SEQ ID NO: 1) of the α transcript of 17.A2 (or 23.A1) of the cloned Ts cells #17.2 (or #23.32). The asterisk (*) refers to the highly conserved residues in V_H, V_L, Vβ and Vα regions.

Figure 3 contains a comparison of deduced amino acid sequences of the TCR α chain transcripts of cloned 17.A2 (SEQ ID NO: 2) and 23.A1 (SEQ ID NO: 3) cDNAs with those of P14.A1 (SEQ ID NO: 4) and human Vα3.1 (SEQ ID NO: 5). The asterisk (*) refer to the highly conserved residues

in the V_H, V_L, Vβ and Vα regions. The symbol '-' designates the residues that are identical to those of cDNA 17.A2.

Figure 4 contains the nucleotide sequences (SEQ ID NOS: 6 and 7) and deduced amino acid sequences (SEQ ID NOS: 8 and 9) for the junctional region of the α chains of TCRs in Ts clones 17.2 and 23.32, aligned with respect to their common Vα15 segment. The highly conserved residues in Jα regions of two clones are marked by asterisks (*). The shaded regions designate the residues of T cell clone #23.32 that differ from those of cloned T cells #17.2, the positions of which are identical.

Figure 5 contains the nucleotide sequences (SEQ ID NOS: 10 and 11) and deduced amino acid sequences (SEQ ID NOS: 12 and 13) for the junctional region of β chains of TCRs of Ts clones. The alignment is based upon the common Vβ8.2 segment. The Ts clone #17.2 utilized Jβ1.1, whereas clone #23.32 utilized Jβ2.5. Spaces are introduced to align the highly conserved sequences among the Jβ regions. The shaded regions designate the residues of clone #23.32 that differ from that of clone #17.2.

Figures 6A, 6B, 6C, 6D, 6E and 6F show down-regulation of OVA-specific antibody responses by TCR peptides. Each group of five Balb/c mice were injected s.c. on day -8, day -6 and day -4 with PBS, or with 25 μg the α-CDR3 peptide or of the β-CDR3 peptide, or with 1:1 mixture (25 μg each) of the α and β CDR3 peptides, in 0.2 ml PBS. On days 1 and 28 all mice were immunized (i.p.) with 20 μg of aggregated OVA (Figures 6A, B and C) or 20 μg of heat-treated HIgG (Figures 6D, E and F), and were bled 15 days after secondary immunization. IgG1 antibody titers were determined by ELISA. Normal mouse serum was used as a negative control and gave an average O.D. readings of 0.05 at a 1:200 dilution. Titration curves for each individual mouse serum are shown. Plots

designated by the filled symbols ●, ▲, ▼, ■, and ◆ represented the results obtained with sera of mice treated with PBS, and the curves with empty symbols ∘, ▵, ▽, □, and ◇ correspond to sera of mice treated with the TCR-peptide(s). Similar results were obtained in three separate experiments. OVA-specific antibody titers of mice treated with PBS (●, ▲, ▼, ■, and ◆) were compared with those of mice (∘, ▵, ▽, □, and ◇) which had been treated with (A) Jα, (B) Jβ, (C) Jα- plus Jβ peptides. HIgG-specific antibodies of mice treated with PBS (●, ▲, ▼, ■, and ◆) were compared with those of mice (∘, ▵, ▽, □, and ◇) which had been treated with (D) Jα, (E) Jβ and (F) Jα- plus Jβ.

Figure 7 shows the immunological response of mice to repeated immunization of allergen. Each group of six Balb/c mice received on day -5 and day -3 either PBS, or 25 μg each of Jα or of Jβ peptide, or mixture of 25 μg each of Jα and Jβ in PBS. On days 1, 28, 63, 93, 138 and 195 all mice were immunized with 20 μg of OVA in PBS. IgG_1 ELISA titers were determined on individual sera on days 43, 51, 77, 84, 107, 114, 159 and 216.

Figure 8 shows the immunological response of mice to allergen challenge. Each group of six Balb/c mice received (intraperitoneally) on day 0, 10 μg of aggregated OVA, and then treated subcutaneously with three courses of PBS alone or, or α-CDR3 or 1:1 mixture of α-CDR3 and β-CDR3 in PBS on days 15, 30 and 45. All mice were immunized (intraperitoneally) with aggregated OVA or heat-treated HIgG in 0.5 ml PBS, on days 52 and 80. Mice were bled 15 days after each immunization and IgG1 titers were determined for individual serum by ELISA. Each bar represents mean titer ±SD.

Figures 9 shows the long lasting in vivo suppression of OVA-specific IgE antibody response by pretreatment with Jα/Jβ peptides of the TCR of OVA-Ts cells #17.2. Each group of five Balb/c mice received (s.c.) on day -8,

day -6 and day -4 either PBS, or 25 μg each of Jα or Jβ pentadecapeptide, or a mixture of 25 μg each of the Jα and Jβ peptides in PBS. On days 1, 28, 63, 93, 138 and 195 all mice were immunized (i.p.) with 20 μg of Fr-I OVA in PBS (as indicated by arrows), and the PCA titers of OVA-specific IgE antibodies of pooled sera were determined, one week after immunizations, i.e., on days 35, 70, 100, 145 and 202 by 24-hr PCA-reaction in hooded rats. Each PCA titer represents the inverse of the highest dilution of the serum, still giving a clearly visible skin reaction (> 5mm in diameter). o:PBS treatment; •: Jα treatment; ▲: Jβ treatment; □: Jα plus Jβ treatment.

Figure 10 shows the antigen-specific abrogation of an established IgE-antibody response by TCR peptides. Each group of four Balb/c mice received (i.p.) 10 μg of FR-I OVA on day 0, and was then treated s.c. with three courses of PBS alone or, Jα or a mixture of Jα and Jβ in PBS on days 15, 30 and 45. All mice were re-immunized (i.p.) with Fr-I OVA in 0.5 ml PBS, on days 52 and 80, and were bled 15 days after each immunization; the IgE titers of pooled sera were determined by the 24-hr PCA reaction. Similar results were obtained in a second experiment. Each course consisted of three s.c. injections of 25 μg of Jα or a mixture of 25 μg of Jα and Jβ in 0.2 ml PBS, administered on alternate days. Mice were injected with OVA on days 52 (secondary response) and 80 (tertiary response). OVA specific IgE titers were determined on day 66 (A) and day 94 (B). Black bars, PBS treatment; hatched bars, Jα treatment; doubly crosshatched bars, Jα+Jβ treatment.

GENERAL DESCRIPTION OF INVENTION

As described above, the present invention relates to a procedure for the induction of tolerance to specific antigens by the treatment of a host with a peptide segment of the TCR α and/or β chain(s) of Ts cells,

particularly induced in vivo by tolerogenic Ag-mPEG conjugates.

As described in more detail in the Examples below, the cDNAs encoding the α and β chains of TCRs of cloned Ts cells specific for (i) ovalbumin (OVA) and (ii) human monoclonal (myeloma) IgG (HIgG) were produced by polymerase chain reaction (PCR). Analysis of the Vα genes indicated that these clones utilized a new member of the Vα15 gene family, which was productively joined to Jα genes that were different for the Ts cells of the two distinct specificities. Sequence analysis of the β chain cDNAs of the two Ts cell clones revealed that, whereas the Vβ8.2 gene was utilized by both clones, the Jβ gene of the OVA-specific Ts clone differed from that of the HIgG-Ts clone. It is inferred that a strictly limited repertoire of TCR genes, comprising the Vα15 and Vβ8.2 rearranged genes, encoded the TCRs of the cloned Ts cells.

Pretreatment of mice with a mixture of pentadecapeptides, comprising the Vα15 chain including the Jα region (residues 95 to 109) of the TCR of the OVA-specific 17.2 Ts cells, downregulated the immune response specific to OVA, but not to HIgG. Moreover, injection of mice with the peptide of this Jα region alone, suppressed >90% of the Ab response to OVA, whereas the Jβ region peptide downregulated Ab production by only 70%. TCR peptides suppressed OVA-specific IgG_1, IgG_{2a}, IgE and total Ig antibody responses.

Furthermore, treatment of mice with a single course of the synthetic peptide(s) corresponding to the CDR3 region(s) of TCR α and β chains of OVA Ts cells, led to the immunosuppression of these mice for >200 days with respect to their potential to mount an anti-OVA antibody response. Moreover, established OVA-specific antibody production was abrogated following peptide vaccination. The immunosuppression induced by these TCR peptide(s)

involved $CD4^+$ T cells, which were necessary but not sufficient. These findings demonstrate the utility of vaccination with TCR peptides of appropriate T cells for the downregulation of antigen-specific immune responses. This method provides a novel therapeutic approach for downregulation of aberrant immune responses, as exemplified by allergies and autoimmune diseases.

Accordingly, this invention provides a method for inducing the antigen specific downregulation of the immune response by administration of peptides designed on the basis of the amino acid sequences of α and β chains of TCR of Ts cells, induced by the corresponding Ag-mPEG conjugates. The present invention may serve as a method directed toward the treatment of diseases and abnormalities which are characterized by the synthesis of unwanted antibodies to specific antigens, such as IgE antibodies responsible for common forms of IgE-mediated allergies.

This method is also useful for the treatment of a broad spectrum of immunological diseases including (a) diseases resulting in aberrant immune responses, such as autoimmune disorders, and (b) malignacies and organ transplant operations, requiring the injection of xenogenic and/or immunogenic, biologically and therapeutically active molecules, such as (i) xenogenic or engineered Abs, including human monoclonal antibodies (moAbs), and immunoconjugates of different Abs or their fragments, (ii) ribosome-inactivating proteins(RIP), which are used as conjugates with cell targetting Abs or Ag-binding fragment thereof, referred to as Immunotoxin (ITX) or "magic bullets", and (iii) biological response modifiers synthesized by immortalizing their progenitor cells or by genetic engineering, which upon their administration may produce unwanted Abs which reduce their efficacy.

The results of the experiments contained in the Examples below demonstrate that pretreatment of mice with TCR peptides results in significant reduction in OVA-specific response, with respect to all isotypes, including IgE antibodies. Furthermore, the injection of these peptides into mice, which were preimmunized and had an established antibody responses, abrogated >90% of their secondary and tertiary IgG_1 and IgE antibody responses.

Preparation and Use of Composition for Treating Allergic Individuals

Compositions, suitable to be used for protecting allergic individuals from developing an allergic reaction or for ameliorating an existing allergic condition, or other immunotherapeutic treatment as described herein, may be prepared from the peptides disclosed herein. Compositions may be prepared as injectables, as liquid solutions or emulsions. The peptides may be mixed with pharmaceutically-acceptable excipients which are compatible with the peptides. Excipients may include, water, saline, dextrose, glycerol, ethanol, and combinations thereof. The composition may further contain minor amounts of auxiliary substances, such as wetting or emulsifying agents, pH buffering agents, or adjuvants to enhance the effectiveness thereof. Methods of achieving an adjuvant effect for the compositions includes the use of agents, such as aluminum hydroxide or phosphate (alum), commonly used as 0.05 to 0.1 percent solution in phosphate buffered saline. Compositions may be administered parenterally, by injection subcutaneously or intramuscularly. Alternatively, other modes of administration including suppositories and oral formulations may be desirable. For suppositories, binders and carriers may include, for example, polyalkylene glycols or triglycerides. Oral formulations may include normally employed incipients, such as, for

example, pharmaceutical grades of saccharine, cellulose, magnesium carbonate and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10 to 95% of the allergen fragment analogs and/or peptides.

The compositions are administered in a manner compatible with the dosage formulation, and in such amount as is therapeutically effective immunotherapeutic treatment of interest. The quantity to be administered depends on the subject to be treated, including, for example, the capacity of the individual's immune system to synthesize antibodies. Precise amounts of peptide required to be administered depends on the judgement of the practitioner. However, suitable dosage ranges are readily determinable by one skilled in the art and may be of the order of nanograms to micrograms of the peptides. Suitable regimens for initial administration and booster does are also variable, but may include an initial administration followed by subsequent administrations. The dosage of the composition may also depend on the route of administration and will vary according to the size of the host.

Immunoassays

The peptides of the present invention are useful as antigens in immunoassays including enzyme-linked immunosorbent assays (ELISA), RIAs and other non-enzyme linked antibody binding assays or procedures known in the art for the detection of allergen specific IgE antibodies. In ELISA assays, the peptide is immobilized onto a selected surface, for example, a surface exhibiting a protein affinity, such as the wells of a polystyrene microtiter plate. After washing to remove incompletely adsorbed peptide, a nonspecific protein, such as bovine serum albumin (BSA) or casein that is known to be antigenically neutral with regard to the test

sample, may be bound to the selected surface. This allows for blocking of nonspecific adsorption sites on the immobilizing surface and thus reduces the background caused by nonspecific bindings of antisera onto the surface. It is understood, however, that a mixture of peptides may be used, either as an immunogen in a composition or as a diagnostic agent.

The immobilizing surface is then contacted with a sample, such as clinical or biological materials to be tested, in a manner conducive to immune complex (antigen/antibody) formation. This may include diluting the sample with diluents, such as solutions of BSA, bovine gamma globulin (BGG) and/or phosphate buffered saline (PBS)/Tween. The sample is then allowed to incubate for from 2 to 4 hours, at temperatures, such as of the order of 25° to 37°C. Following incubation, the sample-contacted surface is washed to remove non-immunocomplexed material. The washing procedure may include washing with a solution, such as PBS/Tween, or a borate buffer.

Following formation of specific immunocomplexes between the test sample and the bound peptide, and subsequent washing, the occurrence, and even amount, of immunocomplex formation may be determined by subjecting the immunocomplex to a second antibody having specificity for the first antibody. If the test sample is of human origin, the second antibody would be an antibody having specificity for human IgE or IgG antibodies. To provide detecting means, the second antibody may have an associated activity, such as an enzymatic activity that will generate, for example, a color development upon incubating with an appropriate chromogenic substrate. Quantification may then achieved by measuring the degree of color generation using, for example, a visible spectra spectrophotometer.

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitations. Immunological and recombinant DNA methods may not be explicitly described in this disclosure but are well within the scope of those skilled in the art.

EXAMPLES

EXAMPLE 1

This Example illustrates the amplification, cloning and sequencing of TCR α and β chain genes of Ts cells.

Materials and Methods:

Ts cell clones. The generation of the HIgG-specific clone #23.32 and of the OVA-specific clone #17.2 were described previously (refs. 61, 63).

RNA isolation. RNA was isolated by modification of the method of Chirgwin et al (ref. 65). Briefly, Ts cells were lysed in 4M guanidinium isothiocyanate, 0.5% Na-lauroyl sarcosine, 7.35% Na-citrate (pH7.0) and 0.1M β-mercaptoethanol. The lysate was overlayed on a cushion of 5.7M CsCl/0.1M EDTA (pH7.5) and the samples were centrifuged at 25,000 rpm (100,000 xg) at 20°C for 22 hrs. The RNA pellets were washed in 70% ethanol and then dissolved in TE (10 mM Tris, pH 8, 1 mM EDTA) at 65°C. The RNA was precipitated in 0.2M sodium acetate and absolute ethanol, and redissolved in water.

RNA-PCR. The RNA was heated to 70°C for 10 minutes in the presence of oligo(dT) or gene specific primer, and was cooled down quickly on ice. The single stranded (ss) cDNA was synthesized using 20 units of M-MLV (H^-) reverse transcriptase (Superscript, Gibco, BRL) (ref. 66) in 5 fold concentrated reaction buffer (250 mM Tris-HCl, pH

8.3, 375 mM KCl, 15 mM $MgCl_2$), 20 mM DTT and 0.5 mM dNTP mixture. For the PCR Taq DNA polymerase (Gibco, BRL) was used (ref. 67). For the amplification of TCR $\alpha\beta$ chains, the corresponding cDNAs were obtained employing oligo(dT) as primer. Amplification of transcripts encoding the β chain was achieved with the 5′ end of Vβ8 and the 3′ end of Cβ (CβE_3) oligonucleotides (Table 1a - the various Tables appear at the end of the disclosure. SEQ ID NOS: 20 to 29). The transcripts encoding α-chains were amplified using the 5′ end of Vα15 and the 3′ end of Cα (CαE_3) oligonucleotides. Each cycle consisted of 1 min at 94°C, 2 min at 55°C and 1 min at 72°C.

Anchor PCR. To determine the 5′ end of the α chain, i.e. Vα region, we followed the overall strategy summarized in Figure 1 and described else where (ref. 68). Total RNA was reverse-transcribed with CαE_3 oligonucleotide, to generate a (-) strand of cDNA utilizing Superscript as the enzyme. Excess of primer was removed by Quagene column. A poly(A) tail was added using terminal deoxynucleotidyl transferase (Gibco, BRL) as per manufacturer's instructions. The single stranded (ss) cDNA was then annealed with the dT_{17} adapter primer and extended to generate the (+) strand. Subsequently, a gene specific internal primer, selected from the 5′ end of Cα , i.e. Cα5RT, and adapter primers were used for amplification and generation of double stranded (ds) DNA.

Cloning of PCR products. PCR products were cloned using the TA cloning system (In Vitrogen, CA), which takes advantage of the A-overhangs of amplified cDNAs that are used to insert the PCR product into a spatially designed vector, namely pCR 1000, providing single 3′T-overhangs at the insertion site. The amplified PCR products obtained as described above were directly ligated onto the pCR 1000 vector, in a 1:2 ratio of vector to insert.

The ligation products were transformed in *E.coli* strain DH5αF′ lacIq$^-$ as described elsewhere (ref. 69).

Briefly, the frozen competent cells were thawed on ice for 30', incubated with plasmids for 30', then heat shocked for 2' at 42°C and then kept for 2' on ice. The mixture was then grown in SOC medium (2% Bacto tryptone, 0.5% Bacto yeast extract, 10mM NaCl, 2.5 mM KCl, 10mM $MgCl_2$, 10mM $MgSO_4$ and 20mM glucose) at 37°C for 1 hr and spread on LB agar plates containing kanamycin (50 μg/ml) and 25 μl of X-gal (40mg/ml). The colonies were allowed to grow for 40 hrs. and the white colonies were picked up and analysed by plasmid isolation. At least 10 transformant colonies were analysed from each of two independent PCR amplifications to ensure the reproducibility of this procedure.

Plasmid DNA isolation. Plasmid DNA was isolated by a modification of the protocol of Birnboim and Doly (ref. 70). Briefly, the bacterial cells were lysed in a solution of 1:2:1 ratios of TE, lysis buffer (1.25% SDS, 0.2N NaOH), and 10M NH_4OVAc. The supernatant was precipitated by absolute EtOH. RNA was removed by digestion with DNAse-free RNAse and the protein was removed by digestion with proteinase K. The DNA was then extracted with phenol, phenol/choloroform and chloroform and precipitated with NaOVAC and two fold EtOH. Inserts were excised by digestion with Eco RI and Hind III restriction endonucleases and checked by electrophoresis in an 1% agarose gel. The plasmids containing the inserts were chosen for further analysis.

DNA sequencing. DNA sequencing was performed employing ds DNA cycle sequencing system (BRL) as per manufacturer's instruction. Polylinker of TA vector was flanked by T_7 promoter and M13 forward primer regions. Therefore, positive recombinants were sequenced directly using T_7 and M13 forward primers. Furthermore, internal gene specific primers of Cα and Cβ regions were used to sequence the entire TCR α/β genes. Each of the amplifications was repeated at least twice with different

batches of mRNA of each of the Ts cell clones. At least 5 clones from each batch of transformants were sequenced to avoid PCR amplification and cloning induced artifacts.

Results

Amplification, cloning and sequencing of TCR α chain genes of Ts clones.

To elucidate the V genes that encode the TCR α chains of the Ts clones of #17.2 and #23.32, Southern blots containing gene fragments of 11 individual cloned Vα genes were hybridized first with the ss cDNA of the #23.32 and #17.2 Ts cells. No hybridization was detected with any available Vα gene probes (data not shown). Therefore, an anchor PCR procedure was employed for the determination of the α chains utilized by these Ts cell clones. A summary of the procedures and primers involved is shown schematically in Figure 1. The ss cDNAs were synthesized from mRNAs of Ts clones #17.2 and #23.32 which were then tailed with poly-(A) and amplified. Transcripts of 540 bps were generated which were gel purified and cloned in pCR1000 vector. Several cDNAs were isolated from the #17.2 and #23.32 Ts cells. Individual cDNAs were sequenced using T_7 and M13 forward primers. It is to be noted that each of the amplifications was repeated at least twice with different batches of mRNA of the cloned Ts cells. In Figure 2 is illustrated the nucleotide sequence (SEQ ID NO: 1) of the Vα gene utilized by cDNAs 17.A2 and 23.A1, which were derived, respectively, from mRNAs of Ts cell clones #17.2 and #23.32. Both cDNAs possessed identical nucleotide sequences.

A unique family of rearranged Vα genes encode TCR of Ag(mPEG)$_n$ induced Ts cells.

In Figure 3 are shown the deduced amino acid sequences (SEQ ID NOS: 2 and 3) of the Vα chains of 17.A2 and 23.A1 Ts cells which have been aligned with respect to the corresponding sequence of a previously reported

partial and non-functional cDNA clone p14A.1 (SEQ ID NO: 4), which has been considered to represent Vα15 (ref. 34). It is to be noted that the V regions of the α chain of the TCR of #17.2 (or #23.32) Ts cells are homologous to the extent of 82% with the sequence of P14A.1 over a span of 81 amino acid residues. Furthermore, P14A.1 did not yield a rearranged transcript because it lacked an open reading frame. These results suggest that the Vα genes of the cloned Ts cells are distinct and may represent a new member of the Vα 15 family; moreover, this novel family of murine Vα genes was homologous to the extent of ~71% with respect to the sequence of the human Vα3.1 gene (ref. 35) (SEQ ID NO: 5).

CDR3 in TCR α chain of Ts cell clones:

The Jα genes utilized by Ts clones #17.2 and #23.32 are shown in Figure 4 (SEQ ID NOS: 6 to 9). From these data it may be concluded that the diversity of the TCR α chains of these Ts clones was confined to a single Vα and two Jα genes. The OVA-specific cloned Ts cells expressed Vα15/Jα 17.A2, whereas the HIgG-specific Ts cells expressed Vα15/Jα 23.A1. The highly concordant alignment of the 3' ends of their common Vα15 gene and of the residues conserved in their Jα regions indicated that the putative TCR-CDR3 loops of the α chains of these Ts cells differed in only 9 distinct residues. Finally, the Cα region of each of these Ts cells was sequenced using $C\alpha E_1$ and $C\alpha E_3$ primers. Two bp changes at positions 684 (i.e. C --> T) and 768 (i.e. G --> C) were found in the Cα chain of each of the cloned Ts cells (data not shown). However, these changes would not lead to any alteration in amino acid residues; this observation is consistent with the findings of Palmer et al (ref. 36).

Primary structures of TCR β chain genes of the Ts cell clones.

To identify the V genes that encode TCR β chains of Ts cell clones, Southern blots containing cloned gene

fragments of 15 individual Vβ genes (kindly provided by Dr. E. Palmer, Colorado) were hybridized with the ss cDNA of Ts cells # 23.32. The results demonstrated that the 23.32 cDNA hybridized only with Vβ8 genes (data not shown). In order to establish the member of Vβ8 gene utilized in each of our Ts clones, the mRNA of Ts clones #23.32 and #17.2 were subjected to RNA-PCR. The ss cDNAs were synthesized from total RNAs using oligo(dT) as primer. The cDNAs were then amplified utilizing the 5′ end of Vβ8 (a consensus primer common to Vβ 8.1, 8.2 and 8.3) and 3′ end of Cβ primers, $C\beta E_3$. An 850 bp transcript was generated from each of these Ts cells. The amplified products were cloned directly into pCR1000 vector (In Vitrogen, CA). Upon screening, several cDNAs were isolated and sequenced using T_7/M13 primers. Primer specific to 5′ end of Cβ, i.e. Cβ5RT, was used to sequence the Dβ-Jβ regions of these cDNAs.

In Figure 5 is shown the alignment of junctional regions of the Vβ-Dβ-Jβ sequences of the two cloned Ts cells (SEQ ID NOS: 10 to 13). Comparison of sequences with the data bank revealed that both Ts cell clones utilized Vβ8.2 (ref. 37). The OVA-specific Ts cell clone #17.2 expressed the Vβ8.2-Dβ1.1-Jβ1.1 gene, whereas the HIgG specific clone #23.32 expressed the Vβ8.2-Dβ1.1-Jβ2.5 gene. The Dβ segments in each set were identical except for 1 or 2 N-region additions at the 3′ end. Alignment of Vβ 8.2 segment and of the residues common to both Jβ1.1 and Jβ2.5 segments revealed that the nucleotide differences between the putative TCR-CDR3 loops of the β chains of OVA- and HIgG-specific Ts clones resided in only 9 residues within the junctional region (Figure 5). It is to be noted that the two Cβ specific primers, $C\beta E_1$ and $C\beta E_3$, were used to sequence the Cβ region of each of these Ts clones; it was established that while the Ts clone #23.32 utilized the Cβ2 gene, clone #17.2 utilized the Cβ1 gene.

EXAMPLE 2

This Example illustrates induction of Ag-specific suppression of the immune response in vivo with TCR α peptides.

Materials and Methods

Animals. Six to eight-week old female BDF1 and Balb/c mice were purchased from Central Animal Care Services of the University of Manitoba.

Antigens and peptides. OVA (5X crystallized) was purchased from ICN Pharmaceuticals (Montreal, PQ); HIgG was isolated from the serum of a myeloma patient (PK) by ammonium sulfate precipitation and ion exchange chromatography on DEAE (ref. 71). Three pentadecapeptides (95% pure), representing the segments of the TCR α chain of the Ts cell #17.2, were synthesized by the Alberta Peptide Institute, Edmonton, AL. Peptides #1 and #2, respectively, (The identification of the peptides employed is shown in Table 1b), corresponding to CDR1 and CDR2 segments of the Vα region, which were common to both clones #17.2 and #23.32, consisted of amino acid sequences, EDVTMNCSYKTYTTV (SEQ ID NO: 14) and YRQKSGKGPAQLILI (SEQ ID NO: 15); peptides with the sequences GSNAKLTFGKGTKLS (SEQ ID NO: 16) and YSNNRLTLGKGTQVV (SEQ ID NO: 18) encompassed, respectively, the Jα regions representing CDR3 segment of the α chains of the OVA-specific and HIgG specific T s cells. The peptide with the amino acid sequence GPNTEVFFGKGTRLTV (SEQ ID NO: 17) of the Jβ region represented the CDR3 segment of the β chain of the OVA-specific Ts cells. An unrelated peptide MIEKINVGFKAAVAAAGGVP (SEQ ID NO: 19) was synthesized and used as control peptide.

Determination of in vivo suppressive activity of TCR α chain peptides.

To establish in vivo the possibility that the suppressive activity of the TCR α chain resides in one or

more of the segments of this chain, three groups of 6 BDF1 mice each received on day 0 one s.c. injection of 0.1 ml of PBS, or 0.1 ml of PBS containing one of the two mixtures , A or B, consisting, respectively, of 25μg or 50 μg of each of the three pentadecapeptides. After 24 hours, each mouse was immunized i.p. with a mixture of 20 μg of OVA and 20 μg of heat-treated HIgG (ref. 71). All the mice were bled 7 and 14 days later and the corresponding total Ig and IgG1 Ab titers of each serum were determined by ELISA. Briefly, the plates were coated with 10 μg of either of OVA or HIgG in PBS, blocked with 3% of bovine serum albumin in PBS and incubated overnight with each individual mouse serum. The plates were washed, treated successively with biotinylated goat anti-mouse Ig or IgG1 Ab (Zymed, Mississagua, ON) and alkaline phosphatase-streptavidine conjugate (Zymed, Mississagua, ON) and finally developed with p-nitrophenyl-phosphate buffer prior to recording their O.D. readings at 405nm.

Results

Induction of antigen-specific suppression by pretreatment of mice with TCRα chain peptides

The immunoregulatory role of the α chain in vivo was examined by injecting a mixture of three pentadecapeptides corresponding to segments 18 to 32, 36 to 50 and 95 to 109 of the TCR α chain of the OVA-specific Ts cell into mice in two different doses. These results illustrated in Table 2 demonstrate that (i) marked OVA-specific suppression of IgG Abs and of IgG1 Abs had been induced by days 7 and 14, respectively, by the mixtures A and B of the three pentadecapeptides, and (ii) the suppression was dose-dependent, i.e., the same mixture of peptides did not significantly affect the HIgG-specific response. Moreover, similar results were obtained in a replicate experiment.

It is to be noted that the TCR α chain of HIgG-Ts cell possesses sequences identical to peptides #1 and #2, and that it differs from the TCR α chain of OVA-Ts cell in the composition of the CDR3 peptide (i.e. peptide #3). Therefore, it was concluded that the difference of the effect of the mixture of the three peptides on the anti-OVA and anti-HIgG Ab responses was due to peptide #3, which represnted the TCR Jα segment. For the confirmation of this conclusion, mice were immunized on days -5 and -3 with 25μg each of the Jα or of the control peptide followed by challenge with OVA or HIgG on day 0 and bleeding on day 14 and 21. As is evident from the results of Table 3, the Jα peptide alone induced 90% suppression of the OVA-specific Ab response without affecting the anti-HIgG Ab response. These results were duplicated in a further experiment. Taken together, these results indicate that, at least, for the OVA-specific Ts cells generated in our system, the Jα peptide of the TCR of these cells was responsible for mediating the suppression of the OVA-specific immune response.

To examine the generality that the CDR3 peptide of the Ts cells specific for a given Ag was endowed with the corresponding capacity to induce Ag-specific suppression in another experiment, mice were pretreated with the pentapeptide corresponding to CDR3 region of the HIgG-Ts cells, and received 7 days later, a s.c. injection of OVA or HIgG and their IgG, Ab titers were determined 10 and 20 days later. As shown in Table 4, the CDR3 peptide of the TCR α chain of HIgG-Ts cells induced suppression in the order of 80% of IgG1 Ab responses specific for HIgG, but not for OVA, as determined on days 10 and 20 after immunization. All these results taken together lead to the conclusion that the CDR3 peptide of the TCR α chain of Ts cells, generated by Ag$(mPEG)_n$ conjugates, was responsible for the observed Ag-specific suppression of the immune response.

The experimental protocol described above was used to examine the effect of J-encoded peptides of the TCR α and β chains, and the possibility of a synergistic effect of both peptides;. As documented by the results presented in Table 5, maximal suppression (>90%) of the anti -OVA IgG_1 titers both on day 14 and 21 were due to the TCR Jα peptide; however, the TCR Jβ peptide also induced significant (about 76%) suppression of the anti-OVA IgG_1 titers. The combined injection of Jα and Jβ peptides did not result in enhanced suppression in relation to that induced by Jα peptide alone. Hence it is concluded that (i) both Jα and Jβ peptides suppressed the anti-OVA Ab responses without affecting significantly the anti-HIgG response, and (ii) that the Jα peptide was more immunosuppressive than the corrresponding Jβ peptide.

EXAMPLE 3

This Example illustrates suppression of secondary anti-OVA Ab responses by TCR peptides of OVA-specific Ts cells 17.2.

To establish if secondary Ab responses to Ag can be suppressed by treatment of mice with TCR peptides of OVA-Ts cells, Balb/c mice were treated with 25 μg each of the Jα or of Jβ peptide referred to above or with a mixture of 25 μg each of them in PBS and the control mice were treated with PBS. On day 1 and day 28, all mice were immunized with 20 μg of OVA or 20 μg of heat-aggregated HIgG as control. The total Ab titers on day 35, 43 and 51 are shown in Table 6. The Ab titers were significantly suppressed in mice treated with peptide(s) in relation to those of control mice. The highest suppression (>90%) was induced by the treatment with the Jα peptide; the Jβ peptide also induced specific suppression (70%) of Ab responses; the suppression induced by a mixture of Jα and Jβ peptides did not exceed that of the Jα peptide alone. In Figures 6A to 6F are shown the anti-OVA IgG_1 titers of sera of mice treated as

described for Table 6. In this case, the highest suppression of OVA-specific titers was seen in mice treated with either Jα or a combination of Jα and Jβ peptides; the mice treated with Jβ peptide also showed suppression 60-70 of OVA-specific IgG_1 titers. Significant enhancement of titers was seen in mice immunized with HIgG in comparison with that of the PBS treated controls. Table 7 demonstrates the OVA-specific IgG_{2a} titers in the same mice. The reduction of IgG_{2a} titers was similar to that of the IgG_1 titers; highest suppression was seen in case of Jα/Jβ and Jα peptide treatment and intermediate level of suppression of response was seen by treatment with Jβ peptide.

In the Jα treated mice which were challenged with HIgG used as a control antigen, showed no reduction of HIgG titers. On the other hand, the mice injected with Jβ or Jα/Jβ peptides exhibited suppression of HIgG-specific IgG_{2a} titers. Taken together, the results of these experiments revealed that pretreatment of mice with TCR peptides induced suppression of secondary immune response; highest suppression was seen by Jα peptide and partial suppression was seen by the Jβ peptide, whereas Jα plus Jβ peptide treatment induced suppression similar to that of Jα.

EXAMPLE 4

This Example illustrates the long lasting tolerance of OVA-specific antibody responses by pretreatment of mice with TCR peptide(s).

Method:

Each group of six Balb/c mice were injected subcutaneously on day -5 and day -3 either PBS, or 25 μg each of α-CDR3 peptide or of β-CDR3 peptide, or 1:1 mixture (25 μg each) of α plus β-CDR3 peptides, in 0.2 ml PBS. On days 1, 28, 63, 93, 138 and 195 (as is indicated by arrow), all mice were immunized i.p. with 20 μg of aggregated OVA in PBS. Mice were bled 15 days

after each immunization. IgG1 antibody titers for individual serum were determined by ELISA. Each point represents the mean of the titers ±SD. Concordant results were obtained in a second experiment. The differences between the OVA-specific titers of mice treated with the α-peptide (or α+β peptides) and the corresponding titers of mice treated with PBS were statistically significant ($P<0.01$).

Results:

The results of prior vaccination with the TCR-CDR3 peptide resulted in persistent immune tolerance at the level of production of specific antibodies is shown in Figure 7. Remarkably, whereas treatment with a single course of the TCR-CDR3 peptide induced tolerance of OVA-specific IgG1 antibody production which was maintained for more than 200 days (as long as it was examined), even after six intermittent injections of OVA (Figure 7), the HIgG-specific antibody titers remained unaffected during this period. The magnitude of the decline in antibody titers induced by different CDR3 peptides was similar to that seen in the above experiment.

EXAMPLE 5

This Example illustrates abrogation of established immune response by TCR peptides.

Method:

Each group of six Balb/c mice received (intraperitoneally) on day 0, 10 μg of aggregated OVA, and then treated subcutaneously with three courses of PBS alone or, or α-CDR3 or 1:1 mixture of α-CDR3 and β-CDR3 in PBS on days 15, 30 and 45. All mice were immunized i.p. with aggregated OVA or heat-treated HIgG in 0.5 ml PBS, on days 52 and 80. Mice were bled 15 days after each immunization and IgG1 titers were determined for individual serum by ELISA. Each bar represents mean titer ±SD. Concordant results were obtained in second experiment. Each course consisted of three subcutaneous

injections of 25 μg of α-CDR3 or 25 μg each of α plus β-CDR3 in 0.2 ml PBS, on alternate days. (A) and (C) mice were challenged with OVA on days 52 and 80. OVA specific IgG1 titers were determined on day 66 (A) and day 94 (C). Differences between OVA-specific titers of mice treated with α plus β-CDR3, and the corresponding titers with PBS were statistically significant ($P<0.01$).

Results:

The ability of the above TCR peptides to tolerize established immune responses was examined and the results are shown in Figure 8. Mice first were immunized with OVA and treated with three courses beginning day 15 with PBS or TCR-CDR3 peptide(s) of either α chain alone or a combination α plus β chains. Mice were challenged on days 52 and 80 with either OVA or HIgG. Examination of the OVA-specific IgG1 and IgE (data not shown) titers on days 66 and 94 revealed that the antibodies specific to OVA were reduced in treated group in comparison with the PBS-treated mice (Figure 8). Remarkably, the peptides varied in the magnitude of tolerance induction inasfar that α+β chain CDR3 peptides induced a higher degree of tolerance (>95%) than the α-CDR3 peptide alone. The immune response to HIgG remained unaffected.

EXAMPLE 6

This Example illustrates in vivo suppression of OVA-specific IgE Ab response by TCR peptides of OVA-Ts cells.

Method:

Ovalbumin, used in this study as a model antigen, constitutes 58% of the whole egg white and plays also the role of a major allergen in individuals allergic to eggs. Thus, OVA has been shown to induce antibodies of both IgG and IgE isotypes (ref. 73). Antibodies of the IgE class are elicited in response to exposure to occupational, environmental and food allergens in genetically predisposed individuals (comprising about 20% of the population). Earlier investigations had demonstrated

that administration of OVA-mPEG conjugates into mice downregulated their anti-OVA IgE immune response, and that this effect was due to induction of Ts cells. Hence, the effects of vaccination with TCR peptides of these Ts cells on (i) the downregulation of the de novo induction, and (ii) the suppression of established IgE antibody response were investigated in this study.

To examine relevance to IgE-mediated allergies the possibility that treatment with TCR peptides of OVA-Ts cells would also lead to suppression OVA-specific IgE Ab response. Each group of 6 mice were treated at day -5 and day -3 with PBS or with 25 μg each of Jα, Jβ or a mixture of Jα and Jβ in PBS. On days 1, 28, 63, 93, 133 and 195 all mice were immunized with 20 μg of OVA. The IgE Ab titers of individual mice were examined on days 35, 70, 100 and 145 by passive cutaneous anaphylaxis test (47).

Results:

The results, presented in Table 8, demonstrate that pretreatment with Jα peptide induced reduction of Ab titers (>90%). The treatment with Jα and Jβ peptide also induced suppression to the extent of 80% of IgE titers compared to PBS control. However, Jβ peptide treatment induced about 40% suppression specific IgE titers. These results demonstrate that specific IgE titers can also be suppressed by the treatment of TCR peptides. The suppression of IgE response was maintained for longer than 200 days as was the IgG1 response. Furthermore, the peptide treatment also suppressed the ongoing IgE response.

EXAMPLE 7

This Example illustrates downregulation of OVA-specific IgE responses by TCR peptide treatment.

Results:

The results presented in the preceding Examples demonstrated that vaccination with TCR peptides led to

suppression of anti-OVA IgG antibody titers. Similar protocols were used in the experiment described here for establishing if the OVA induced specific IgE antibody response may be downregulated by TCR peptides. Briefly, mice were vaccinated with the pentadecapeptides corresponding to the Jα and/or Jβ regions of TCRs of the cloned OVA-Ts cell prior to initiation of immunizations with repeated injections of OVA at intervals of 4-6 weeks. The IgE antibody titers were determined by the PCA assay in hooded rats.

As shown in Figure 9, the OVA-specific IgE antibody response was suppressed by treatment with Jα and/or Jβ peptides of the TCR of cloned OVA-Ts cells. Remarkably, treatment with a single course of injection of the TCR-CDR3 peptide induced suppression of OVA-specific IgE antibody production for more than 200 days, inspite of six injections of immunogenic OVA. The magnitude of the suppression varied with different peptides. Marked reduction (>90%) in IgE antibody titers resulted upon treatment of mice with the pentadecapeptide corresponding to either Jα or Jα in combination with Jβ peptide; the Jβ peptide alone induced about 50% suppression of IgE antibody production.

EXAMPLE 8

This Example illustrates abrogation of an established allergen specific IgE- antibody response by TCR peptides.

Method:

Each group of six Balb/c mice received (i.p.) 10 μg of OVA on day 0, and were then treated s.c. with three courses of PBS alone or, α-CDR3 or 1:1 mixture of α-CDR3 and β-CDR3 in PBS on days 15, 30 and 45. All mice were re-immunized (i.p.) with OVA or ha-HIgG in 0.5 ml PBS, on days 52 and 80, and were bled 15 days after each immunization; the IgE titers of pooled sera were determined by PCA 24-hr later. Similar results were

obtained in a second experiment. Each course consisted of three subcutaneous injections of 25 μg of α-CDR3 or a mixture of 25 μg of α-CDR3 and β-CDR3 in 0.2 ml PBS, administred on alternate days. As illustrated in panels (A) and (B) mice were injected with OVA on days 52 (secondary) and 80 (tertiary). OVA specific IgE titers were determined on day 66 (A) and day 94 (B).

Results:

The determination of IgE antibody titers by PCA is shown in Figure 10. Clearly, on-going OVA-specific IgE antibodies were suppressed (>90%) by treatment with a mixture of Jα and Jβ peptides, as determined from the secondary (d52) and tertiary (d94) PCA titers. The Jα peptide treatment suppressed only 75%-80% of the established IgE response. Thus, similar to the IgG_1 response, for a more effective suppression of the antigen-specific established IgE antibody production required treatment with Jα and Jβ peptides.

EXAMPLE 9

This Example illustrates downregulation of in vivo antibody responses of primed spleen cells by helper T (Th) cells of TCR-peptide treated group.

Methods:

Group of four Balb/c mice received i.p. on day 0, 10 μg of aggregated OVA, and then treated subcutaneously with three courses of either PBS alone, or 1:1 mixture of α-CDR3 and β-CDR3 on days 15, 21 and 28. On day 35, mice were sacrificed and $CD4^+$ T cells were separated from each group by using polypropylene immunocolumn (collect plus, Biotex Laboratories, Inc.). For control purposes, $CD4^+$ T cells were separated from normal Balb/c mouse spleens. OVA primed spleen cells were isolated 15 days after immunization of mice with 50 μg OVA emulsified in complete Freund's adjuvant (subcutaneously over the hind legs). OVA primed spleen cells were cultured in quadruplate, at two different concentrations with Th

cells separated from mice treated with either PBS, or peptides or naive mouse, at three different concentrations, in the presence of 100 ng/ml of DNP-OVA in complete medium. Forty-eight hours later, the cells were washed and cultured in fresh medium for additional 4 days. The amount of antibody in each well, in the latter culture supernatant, was measured by ELISA using plate coated with OVA, as described above.

Results:

The mechanism underlying the tolerance induced by these TCR-CDR3 peptides was examined. The possibility that the peptide treatment induces production of peptide-specific antibodies was addressed; however, in treated mice no peptide-specific antibodies could be detected. Although this is contrary to some other studies involving EAE, it is to be pointed out that in this study all treatment regimens included peptides in PBS and not with any adjuvant, i.e., CFA. Moreover, the TCR peptide did not bind to OVA directly; thus ruling out the possibility that the TCR peptides directly block the antigen-MHC-TCR interaction leading to reduction of OVA-specific antibody production. The fact that treatment of mice with TCR peptide followed by challenge with OVA-DNP resulted in the downregulation of both OVA- and DNP-specific IgG1 antibody titers, suggests that the peptide treatment downregulates OVA-specific helper T cell response (data not shown). We also examined whether the peptides elicit a T cell response (Table 9). The results clearly demonstrate that the down regulation of specific antibody production by TCR peptides clearly involve peptide-specific $CD4^+$ T cells.

SUMMARY OF THE DISCLOSURE

In summary of this disclosure, the present invention represents a unique approach to immunotherapy. While the description of - the invention contains specific embodiments thereof, it will be understood that it is

capable of further modification and applications to the one skilled in the art of molecular immunology. This description is, therefore, intended to cover any variations, uses or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as would not be anticipated on the basis of known principles and and existing practice within the area to which the invention pertains.

LITERATURE REFERENCES

1. Freidhoff LR. In: Genetic and Environmental factors in clinical allergy, Marsh DG and Blumenthal MN (eds.) Univ. of Minnesota Press (1989).
2. Loca AF and Cooke RA. J. Immunol. 8: 162 (1923).
3. Richter, M. *et al.* J. Allergy 29: 298 (1958).
4. Marsh, DG. *et al.* Immunology 22: 1013 (1972).
5. Lichtenstein, LM *et al.* In: 11th Int. Congr. of Allergology and Clin. Immunol. Kerr Jw and Ganderton MA (eds.) pp. 285 (1983).
6. Hamilton RG. Curr. Opinions in Immunol. 2: 558 (1990).
7. Bousquet J. *et al.* J. Allergy Clin. Immunol. 84: 546 (1989).
8. Creticos PS *et al.* J. Allergy Clin. Immunol. 84: 197 (1989).
9. Kay AB. Clin. Exp. Allergy 19: 591 (1989).
10. CMS Update: Desensitizing vaccines Brit. Med. J. 293: 948 (1986).
11. Ekramoddoullah AKM. *et al.* Int. Arch. Allergy Clin. Immunol. 80:100 (1986).
12. Kahn CR and Marsh DG. Fed. Proc. 41: 826 (1982).
13. Chua KY. *et al.* J. Exp. Med 167: 175 (1988).
14. Chua KY. *et al.* Int. Arch. Allergy Clin. Immunol. 85: 127 (1988).
15. Tovey ER, Johnson MC, Roche AL, Cobon GS, Baldo BA. J. Exp. Med. 170: 1457 (1989).
16. Fang KSY. *et al.* Proc. Natl. Acad. Sci. USA 85: 895 (1988).
17. Breitenader H. *et al.* EMBO J. 8: 1935 (1989).
18. Mohapatra SS. *et al.* Int. Arch. Allergy Appl. Immunol. 91: 362 (1990).
19. King TP. In: Proc. 8th Int. Congr. Allergology Clin. Immunol. Mounro-Ashman (ed.), Elsevier pp. 394 (1974)
20. Roebber M. *et al.* J. Immunol. 131: 706 (1983).
21. Smitt JJ. *et al.* Mol. Immunol. 25: 355 (1988).

22. Ford SA, Baldo BA. Int. Arch. Allergy Appl. Immunol. 81: 193 (1986).
23. Berg L.J. Current Opinion Immunol. 2:87 (1989).
24. Kyewski B. and Huenig T. Immunol. today 13:288 (1992).
25. Miller J.F.A.P. et al Cold Spring Harbour Symp. Quant. Biol. 54:807 (1989).
26. Davis M.M, Bjorkman PJ. Nature 334: 395 (1988).
27. Cothia C. et al EMBO J. 7:3745 (1988).
28. Green D.R. et al. Proc. Natl. Acad. Sci. USA. 88:8475 (1991).
29. Kuchroo V.K. et al. Proc. Natl. Acad. Sci. USA. 88:8700 (1991).
30. Danska J.S. Current Opinion Immunol. 2:81 (1989).
31. Kronenberg M. et al Annu. Rev. Immunol. 4:529 (1986).
32. Winoto A. et al Nature 324:679 (1986).
33. Tan K.N. et al Cell 54:247 (1988).
34. Lai M.Z. et al JEM 168:1081 (1988).
35. Urban J.L. et al Cell 54:577 (1988).
36. Morel P.A. JEM 166:583 (1987).
37. Matis L.A. Ann. Rev. Immunol. 8:65 (1990).
38. Henderson R.A. et al. Science 255:1264 (1992).
39. Panina-Bordignon P. et al. Science 252:1548 (1991).
40. Jorgensen J.L. et al. Nature 355:224 (1992).
41. Kisielow P. et al. Nature 333:742 (1988).
42. Tonegawa S. Plenary lecture, 8th Int. Cong. of Immunology, Budapest 1992).
43. Morahan G. et al. Proc. Natl. Acad. Sci. 88:11421 (1991).
44. Burns F.R. et al. JEM 169:27 (1989).
45. Vanderbark A. et al. Nature 341:541 (1989).
46. Offner H. et al. Science 251:430 (1991).
47. Vanderbark A. Plenary lecture, 8th Int. Cong. of Immunol. Budapest (1992).
48. Reiner S.L. et al. Science 259: 1457 (1993).
49. Renz H. et al.- Proc. Natl. Acad. Sci. USA 89: 6438 (1992).

50. Wedderburn L.R. et al. Proc. Natl. Acad. Sci. 90: 8214 (1993).
51. Mohapatra S.S. et al. Immunology 81: 15 (1994).
52. Adorini L. Immunol. Today 14: 285 (1993).
53. Lee W.Y and Sehon A.H. Nature 267: 618 (1977).
54. Lee W.Y and Sehon A.H. Int. Arch. Allergy appl. Immunol. 56: 193 (1978).
55. Sehon A.H. Prog Allergy 32: 161 (1982).
56. Lee W.Y. Sehon A.H. Int. Arch. Allergy appl. Immunol. 64: 100 (1981).
57. Lee W.Y. et al. Int. Arch. Allergy appl. Immunol. 64: 110 (1981).
58. Wilkinson I. et al. Immunol. Lett. 15: 17 (1987).
59. Wilkinson I. et al. J. Immunol. 139: 326 (1987).
60. Sehon A.H. *In*: Molecular biology and allergy, Eds. A.S.E. Shami and T.G. Merrett, Adv. Biosci. 74:327 (1989).
61. Takata, M. et al. J. Immunol. 145:2846 (1990).
62. Takata, M. et al. Cell Immunol. 137:139 (1991).
63. Chen Y. et al. Cell Immunol. 142:16 (1992).
64. Chen Y. et al. J. Immunol 152:3 (1994).
65. Chirgwin, J.M., A.E. Przybyla, R.J. Macdonald, and W.J. Rutter. Biochemistry. 18:5294 (1979).
66. Kotewicz M.L.et al. Nucl. Acid. Res. 16:265 (1988).
67. Saiki R.K. et al. Science (Wash. DC). 239:487 (1988).
68. Frohman M.A. et al. Proc. Natl. Acad. Sci. USA 85: 8998 (1988).
69. Hanahan, D. J. Mol. Biol. 166:557 (1983).
70. Birnboim, H.C., and J. Doly. Nucl. Acids. Res. 7:1513 (1979).
71. Maiti P.K. et al. Int. J. Cancer Supplement 3:17 (1988).
72. Zhang L.et al. Immunology 76:158 (1992).
73. Hoffman D.J. Allergy Clin Immunol 71: 481 (1983).

TABLE 1a:

LIST OF PRIMERS

PRIMER	SEQUENCE	SEQ ID NOS:
Vβ8	5′ ACATGGAGGCTGCAGTCACCCA 3′	20
Cβ5RT	5′ TGATGGCTCAAACAAGGAGACCTT 3′	21
$C\beta E_1$	5′ AGGATCTGAGAAATGTGACTC 3′	22
$C\beta E_3$	5′ TTTCTTGACCATGGCCATCAGC 3′	23
Vα15	5′ GTCCTAGGAACCAGGTTCCA 3′	24
$C\alpha E_1$	5′ ATCCAGAACCCAGAACCTGCT 3′	25
$C\alpha E_3$	5′ CTCAACTGGACCACAGCCTCA 3′	26
Cα5RT	5′ GAGGGTGCTGTCCTGAGACCG 3′	27
Adaptor	5′ GACTCGAGTCGACATCGA 3′	28
dT17 adaptor	5′ GACTCGAGTCGACATCGATTTTTTTTTTTTTTT 3′	29

TABLE 1b:

LIST OF PEPTIDES

ANTIGEN	TCR CHAIN	PEPTIDE SEQUENCE	SEQ ID NO:
OVA	CDR1-α	EDVTMNCSYKTYTTV	14
	CDR2-α	YRQKSGKGPAQLILI	15
	CDR3-α	GSNAKLTFGKGTKLS	16
	CDR3-β	GPNTEVFFGKGTRLTV	17
HIgG	CDR3-α	YSNNRLTLGKGTQVV	18
Control Peptide		MIEKINVGFKAAVAAAGGVP	19

TABLE 2

In vivo suppression of the OVA-specific Ab response by pretreatment with the mixture of TCR α chain peptides

Treatment[a]	Anti-OVA ELISA titers[b]		Anti-HIgG ELISA titers[b]	
	Total Ig	IgG1	Total Ig	IgG1
PBS	2250 ± 119	3750 ± 830	1300 ± 437	15,840 ± 2670
Peptides (mixture A)	615 ± 495	2662 ± 674	772 ± 91	9,937 ± 3110
Peptides (mixture B)	208 ± 99*	1056 ± 170*[c]	1375 ± 246	21,690 ± 2280

[a] Each group of six BDF1 mice received on day 0 either PBS, or a mixture A and B of the three pentadecapeptides; consisting respectively of 25 and 50 μg of each of the three peptides. One day later all mice were immunized with a mixture of 20 μg of OVA and 20 μg of heat-treated HIgG in PBS.

[b] Total Ig Ab titers on day 7 and IgG1 Ab titers on day 14 were determined for individual sera and the numbers in the Table represent the means ± SDs. Data shown here are from one of two experiments.

[c] The differences in OVA-specific titers of groups treated with PBS or the mixture B were statistically significant ($p<0.01$).

TABLE 3

In vivo suppression of the OVA specific Ab response by pretreatment of mice with the by TCR-Jα peptide of OVA-Ts cells

Treatment[a]	Anti-OVA IgG1 titers		Anti-HIgG IgG1 titers	
	Day 14	Day 21	Day 14	Day 21
PBS	7666 ± 548	18000 ± 1229	41250 ± 5907	16000 ± 2121
Jα-peptide	380 ± 93*[b]	1464 ± 731*	39500 ± 5795	22500 ± 1892
Control peptide	8255 ± 578	19000 ± 988	43500 ± 4598	18900 ± 1272

[a] Each group of six BDF1 mice received on day -5 and day -3 either PBS, or 25 μg each of Jα or of control peptide in PBS. On day 0 all mice were immunized with 20 μg of OVA or 20 μg of heat-treated HIgG in PBS. IgG1 ELISA titers were determined for individual sera on days 14 and 21. Each number represents the mean ± SD. Data shown here from one of two experiment.

[b] The differences in OVA-specific titers of groups treated with PBS or the Jα peptide were statistically significant ($p<0.01$).

TABLE 4

In vivo Suppression of the HIgG Specific Antibody Response by Pretreatment of Mice with the TCR-Jα Peptide of HIgG-Ts cells

Treatment[a]	Anti-HIgG IgG_1 titers[b]		Anti-OVA IgG_1 titers	
	Day 10	Day 20	Day 10	Day 20
PBS	28,000 ± 4508	35,000 ± 5500	6,500 ± 898	15,500 ± 3250
Jα-peptide*	6,000 ± 1051*	8,200 ± 1840	6,800 ± 1050	15,800 ± 5050
Control-peptide	29,250 ± 4895	36,500 ± 5400	7,200 ± 985	16,200 ± 3060

[a] Each group of six BDF1 mice received on three days either PBS, or 50 μg of the Jα or of the control peptide in PBS. Seven days after the last treatment all mice were immunized with 20 μg of either ha-HIgG or OVA in PBS.

[b] The IgG_1 ELISA titers were determined on individual sera 10 and 20 days after immunization. Each number represents the mean of the titers ± SD. Similar results were obtained in a second experiment.

* The differences between the HIgG-specific titers of mice treated with the Jα-peptide and of the mice treated with PBS were statistically significant ($p<0.01$).

TABLE 5
In vivo suppression of the OVA-specific Ab response by the TCR-Jα/Jβ peptides of OVA-Ts cells

Treatment[a]	Anti-OVA ELISA titers		Anti-HIgG ELISA titers	
	Day 14	Day 21	Day 14	Day 21
PBS	8,706 ± 856	19,560 ± 3329	51,250 ± 5808	18,000 ± 2520
Jα-peptide	360 ± 103*[b]	1,556 ± 640*	39,800 ± 8795	25,500 ± 2896
Jβ-peptide	1,600 ± 481	4,766 ± 1542	40,600 ± 6446	28,200 ± 4409
Jα+Jβ peptides	1,033 ± 343	2,991 ± 1165	32,330 ± 3480	23,830 ± 3429

[a] Each group of six Balb/c mice received on day -5 and day -3 either PBS, or 25 μg each of Jα or of control peptide in PBS. On day 0 all mice were immunized with 20 μg of high molecular weight OVA or 20 μg of heat-treated HIgG in PBS. IgG1 ELISA titers were determined on individual sera on days 14 and 21. Each number represents the mean ± SD.

[b] The differences in OVA-specific titers of groups treated with PBS or the Jα-peptide were significant ($p<0.01$). PBS.

TABLE 6

In vivo suppression of total IgG Ab response specific to OVA by TCR-Jα peptide of OVA-Ts cells

Treatment[a]	Anti-OVA ELISA titers			Anti-HIgG ELISA titers		
	Day 35	Day 43	Day 51	Day 35	Day 43	Day 51
PBS	50000	50000	52000	48000	72000	45000
Jα-peptide	2000*[b]	2800*	3200*	62000	72000	60000
Jβ-peptide	16000	18000	22000	48000	60000	60000
Jα+Jβ peptides	2400*	2200*	3000*	80000	50000	70000

[a] Each group of six Balb/c mice received on day -5 and day -3 either PBS, or 25 μg each of Jα or of Jβ peptide or mixture of 25 μg each of Jα and Jβ in PBS. On day 1 and day 28 all mice were immunized with 20 μg of OVA or 20 μg of heat-treated HIgG in PBS. Total IgG ELISA titers were determined on individual sera on day 35,43 1nd 51.

[b] The differences in OVA-specific titers of groups treated with PBS or the Jβ peptide versus the Jα or Jα+Jβ peptides were statistically significant ($p<0.01$).

TABLE 7

In vivo Suppression of IgG_{2a} Ab Response Specific to OVA by TCR-Jα Peptide of OVA-Ts cells

Treatment[a]	Anti-OVA ELISA titers			Anti-HIgG ELISA titers		
	Day 35	Day 43	Day 51	Day 35	Day 43	Day 51
PBS	8000	9000	8500	18000	17000	12000
Jα-peptide	320*[b]	300*	300*	18000	19000	12000
Jβ-peptide	2200	4000	2300	6000	8500	5200
Jα-Jβ peptides	220*	300*	260*	6000	8500	4200

[a] Each group of six Balb/c mice received on day -5 and day -3 either PBS, or 25 μg each of Jα or of Jβ peptide or mixture of 25 μg each of Jα and Jβ in PBS. On day 1 and day 28 all mice were immunized with 20 μg of high molecular weight OVA or 20 μg of heat-treated HIgG in PBS. IgG_{2a} ELISA titers were determined on individual sera on day 35, 43 and 51.

[b] The differences in OVA-specific titers of groups treated with PBS or Jβ peptides versus Jα or Jα+ Jβ peptides were statistically significant ($p<0.01$).

TABLE 8

Long Lasting *In Vivo* Suppression of OVA-Specific IgE Antibody Response by the Pretreatment with TCR Jα/Jβ Peptides of OVA-Ts cells

Treatment[a]	PCA titers			
	d35	d70	d100	d145
PBS	1,280	1,920	7,680	8,000
Jα-peptide	100	120	640	700
Jβ-peptide	640	1,000	2,460	3,500
Jα+Jβ peptides	150	400	640	700

[a] Each group of six Balb/c mice received on day -5 and day -3 either PBS, or 25 μg each of Jα or of Jβ peptide, or mixture of 25 μg each of Jα and Jβ in PBS. On days 1, 28, 63, 93, 138 and 195 all mice were immunized with 20 μg of OVA in PBS. OVA-specific IgE antibodies were determined on pooled sera, one week after each immunization i.e. on days 35, 70, 100 and 145, by 24-h passive cutaneous anaphylaxis (PCA) in hooded rats. PCA titer was calculated as the highest dilution giving a clearly visible skin reaction (> 5mm in diameter).

TABLE 9

Downregulation of *in vitro* antibody responses of primed spleen cells by helper T (Th) cells of TCR-peptide treated group.

		Anti-OVA total Ig titer of cultured supernatant Th cells x 10^6		
Primed spleen cells		0.125	0.250	0.500
1.5 x 10^6	PBS	1040 ± 100	775 ± 80	276 ± 30
	Peptide-treated	190 ± 20	95 ± 10	125 ± 15
	Untreated	850 ± 50	760 ± 80	235 ± 25
1.0 x 10^6	PBS	560 ± 60	453 ± 50	180 ± 20
	Peptide-treated	61 ± 10	100 ± 10	98 ± 10
	Untreated	616 ± 70	700 ± 70	410 ± 45

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: UNIVERSITY OF MANITOBA
(B) STREET: 311 Administration Building
(C) CITY: Winnipeg
(D) STATE: Manitoba
(E) COUNTRY: Canada
(F) POSTAL CODE (ZIP): R3P 2N2
(G) TELEPHONE: (204) 474-8418
(H) TELEFAX: (204) 261-0325

(A) NAME: MOHAPATRA, SHYAM S.
(B) STREET: 364 Lindenwood Drive East
(C) CITY: Winnipeg
(D) STATE: Manitoba
(E) COUNTRY: Canada
(F) POSTAL CODE (ZIP): R3P 2H1

(A) NAME: SEHON, ALEC H.
(B) STREET: 695 Academy Road
(C) CITY: Winnipeg
(D) STATE: Manitoba
(E) COUNTRY: Canada
(F) POSTAL CODE (ZIP): R3N 0E8

(ii) TITLE OF INVENTION: DOWNREGULATION OF ALLERGEN-SPECIFIC IMMUNE RESPONSE

(iii) NUMBER OF SEQUENCES: 29

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 883 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

TTTGCTGTCC TAGGAACCAG GTTCCACTTC AGGGTGCAGC ACAGCCTTTC CTGTGACATC 60

AATAAAGCAA GAAAAATGAA CAGATTCCTG GGAATATCTT TGGTGACTCT ATGGTTTCAA 120

GTGGCCTGGG CAAAGAGCCA ATGGGGAGAA GAGAATCTTC AGGCTCTGAG CATCCAGGAG 180

GGTGAAGATG TCACCATGAA CTGCAGTTAC AAGACTTACA CAACTGTTGT TCAGTGGTAC 240

AGACAGAAGT CAGGCAAAGG CCCTGCCCAG CTAATCTTAA TACGTTCAAA TGAGCGAGAG 300

AAGCGCAGTG GAAGACTCAG AGCCACCCTT GACACTTCCA GCCAGAGAAG CTCCCTGTCC 360

ATCACTGGTA CTCTAGCTAC AGACACTGCT GTGTACTTCT GTGCTACTGG GGGAGGAAGC 420

AATGCAAAGC TAACCTTCGG GAAAGGCACT AAACTCTCTG TTAAATCAAA CATCCAGAAC 480

CCAGAACCTG CTGTGTACCA GTTAAAAGAT CCTCGGTCTC AGGACAGCAC CCTCTGCCTG 540

TTCACCGACT TTGACTCCCA AATCAATGTG CCGAAAACCA TGGAATCTGG AACGTTCATC 600

ACTGACAAAA CTGTGCTGGA CATGAAAGCT ATGGATTCCA AGAGCAATGG GGCCATTGCC 660

TGGAGCAACC AGACAAGCTT CACCTGCCAA GATATCTTCA AAGAGACCAA CGCCACCTAC 720

CCCAGTTCAG ACGTTCCCTG TGATGCCACG TTGACTGAGA AAAGCTTTGA AACAGATATG 780

AACCTAAACT TTCAAAACCT GTCAGTTATG GGACTCCGAA TCCTCCTGCT GAAAGTAGCC 840

GGATTTAACC TGCTCATGAC GCTGAGGCTG TGGTCCAGTT GAG 883

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 92 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Lys Ser Gln Trp Gly Glu Glu Asn Leu Gln Ala Leu Ser Ile Gln Glu
1 5 10 15

Gly Glu Asp Val Thr Met Asn Cys Ser Tyr Lys Thr Tyr Thr Thr Val
20 25 30

Val Gln Trp Tyr Arg Gln Lys Ser Gly Lys Gly Pro Ala Gln Leu Ile
35 40 45

Leu Ile Arg Ser Asn Glu Arg Glu Lys Arg Ser Gly Arg Leu Arg Ala
50 55 60

Thr Leu Asp Thr Ser Ser Gln Ser Ser Ser Leu Ser Ile Thr Gly Thr
65 70 75 80

Leu Ala Thr Asp Thr Ala Val Tyr Phe Cys Ala Thr
85 90

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 92 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Lys Ser Gln Trp Gly Glu Glu Asn Leu Gln Ala Leu Ser Ile Gln Glu
1 5 10 15

Gly Glu Asp Val Thr Met Asn Cys Ser Tyr Lys Thr Tyr Thr Thr Val
20 25 30

```
Val Gln Trp Tyr Arg Gln Lys Ser Gly Lys Gly Pro Ala Gln Leu Ile
        35                  40                  45

Leu Ile Arg Ser Asn Glu Arg Glu Lys Arg Ser Gly Arg Leu Arg Ala
    50                  55                  60

Thr Leu Asp Thr Ser Ser Gln Ser Ser Ser Leu Ser Ile Thr Gly Thr
65                  70                  75                  80

Leu Ala Thr Asp Thr Ala Val Tyr Phe Cys Ala Thr
                85                  90
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
Leu Ser Val His Glu Gly Glu Ser Val Thr Val Asn Cys Ser Tyr Thr
1               5                   10                  15

Thr Ser Ile Thr Ala Leu Gln Trp Tyr Arg Gln Lys Ser Gly Glu Gly
            20                  25                  30

Pro Ala Gln Leu Ile Leu Ile Arg Ser Asn Glu Arg Glu Lys Arg Asn
        35                  40                  45

Gly Arg Leu Arg Ala Thr Leu Asp Thr Ser Ser Gln Ser Ser Ser Leu
    50                  55                  60

Ser Ile Thr Ala Thr Arg Cys Glu Asp Thr Ala Val Tyr Phe Cys Ala
65                  70                  75                  80

Thr
```

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 92 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
Asn Ser Gln Gln Gly Glu Glu Asp Pro Gln Ala Leu Ser Ile Gln Glu
1               5                   10                  15

Gly Glu Asn Ala Thr Met Asn Cys Ser Tyr Lys Thr Ser Ile Asn Asn
            20                  25                  30

Leu Gln Trp Tyr Arg Gln Asn Ser Gly Arg Gly Leu Val His Leu Ile
        35                  40                  45
```

Leu Ile Arg Ser Asn Glu Arg Glu Lys His Ser Gly Arg Leu Arg Val
50 55 60

Thr Leu Asp Thr Ser Lys Lys Ser Ser Ser Leu Leu Ile Thr Ala Ser
65 70 75 80

Arg Ala Ala Asp Thr Ala Ser Tyr Phe Cys Ala Thr
85 90

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

TTCTGTGCTA CTGGGGGAGG AAGCAATGCA AAGCTAACCT TCGGGAAAGG CACTAAACTC 60

TCTGTTAAAT CAA 73

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Phe Cys Ala Thr Gly Gly Gly Ser Asn Ala Lys Leu Thr Phe Gly Lys
1 5 10 15

Gly Thr Lys Leu Ser Val Lys Ser
20

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

TTCTGTGCTA CTCCGGACTA CAGCAACAAC AGACTTACTT TGGGGAAGGG AACCCAGGTG 60

GTGGTGTTAC CAA 73

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Phe Cys Ala Thr Pro Asp Tyr Ser Asn Asn Arg Leu Thr Leu Gly Lys
1 5 10 15

Gly Thr Gln Val Val Val Leu Pro
20

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

TGTGCCAGCG GTGATGCAGG GCCAAACACA GAAGTCTTCT TTGGTAAAGG AACCAGACTC 60

ACAGTTGTA 69

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Cys Ala Ser Gly Asp Ala Gly Pro Asn Thr Glu Val Phe Phe Gly Lys
1 5 10 15

Gly Thr Arg Leu Thr Val Val
20

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

TGTGCCAGCG GTGATGCAGG GTTTAACCAA GACACCCAGT ACTTTGGGCC AGGCACTCGG 60

CTCCTCGTGT TA 72

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Cys Ala Ser Gly Asp Ala Gly Phe Asn Gln Asp Thr Gln Tyr Phe Gly
1 5 10 15

Pro Gly Thr Arg Leu Leu Val Leu
20

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Glu Asp Val Thr Met Asn Cys Ser Tyr Lys Thr Tyr Thr Thr Val
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Tyr Arg Gln Lys Ser Gly Lys Gly Pro Ala Gln Leu Ile Leu Ile
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Gly Ser Asn Ala Lys Leu Thr Phe Gly Lys Gly Thr Lys Leu Ser
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Gly Pro Asn Thr Glu Val Phe Phe Gly Lys Gly Thr Arg Leu Thr Val
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Tyr Ser Asn Asn Arg Leu Thr Leu Gly Lys Gly Thr Gln Val Val
1 5 10 15

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Met Ile Glu Lys Ile Asn Val Gly Phe Lys Ala Ala Val Ala Ala Ala
1 5 10 15

Gly Gly Val Pro
20

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

ACATGGAGGC TGCAGTCACC CA 22

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

TGATGGCTCA AACAAGGAGA CCTT 24

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

AGGATCTGAG AAATGTGACT C 21

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

TTTCTTGACC ATGGCCATCA GC 22

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

GTCCTAGGAA CCAGGTTCCA 20

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

ATCCAGAACC CAGAACCTGC T 21

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

CTCAACTGGA CCACAGCCTC A 21

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

GAGGGTGCTG TCCTGAGACC G 21

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

GACTCGAGTC GACATCGA 18

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

GACTCGAGTC GACATCGATT TTTTTTTTTT TTTTT 35

CLAIMS

1. A synthetic peptide having an amino acid sequence comprising at least a portion of the complementarity determining region 3 (CDR3) of a T-cell receptor (TCR) of an antigen and capable of eliciting a T-cell response.

2. The synthetic peptide of claim 1 wherein said T-cell receptor is a human T-cell receptor.

3. The synthetic peptide of claim 2 wherein said amino acid sequence is encoded by a nucleotide sequence comprising the J-gene of the α-chain of the TCR.

4. The synthetic peptide of claim 2 wherein said amino acid sequence is encoded by a nucleotide sequence comprising the J-gene of the β-chain of the TCR.

5. The synthetic peptide of claim 2 comprising a mixture of such peptides, one encoded by a nucleotide sequence comprising the J-gene of the α-chain of the TCR and another encoded by a nucleotide sequence comprising the J-gene of the β-chain of the TCR.

6. The synthetic peptide of claim 1 which has about 8 to about 12 amino acids and is capable of binding to a class I major histocompatibility complex (MHC) molecule.

7. The synthetic peptide of claim 1 which has about 12 to about 27 amino acids and is capable of binding to a class II MHC molecule.

8. The synthetic peptide of claim 1 bound to a non-immunogenic substrate.

9. The synthetic peptide of claim 8 which said non-immunogenic substrate is selected from polymeric materials.

10. The synthetic peptide of claim 9 wherein said polymeric material is selected from carboxymethyl celluloses, monomethoxypolyethylene glycols and polyvinyl alcohols.

11. The synthetic peptide of claim 1 wherein said antigen is an allergen.

12. An immunosuppressive composition for immunotherapy, comprising at least one synthetic peptide as claimed in any one of claims 1 to 11 and a pharmaceutically-acceptable carrier therefor.

13. The composition of claim 12 further comprising an adjuvant.

14. A method of identifying a peptide having an amino acid sequence comprising at least a portion of the CDR3 region of a T-cell receptor of an antigen and capable of eliciting a T-cell response, which comprises:

effecting induction of regulatory T-cells to a desired antigen,

determining the nucleotide sequence of T-cell receptors of said regulatory T-cells,

determining the portion of said nucleotide sequence of said T-cell receptors which codes for the CDR3 region of said T-cell receptors, and

deducing the amino acid sequence of said determined portion of said nucleotide sequence as a determination of said amino acid sequence of said peptide.

15. The method of claim 14 wherein said regulatory T-cells are induced by administration to a host of a conjugate of a non-immunogenic substrate and said desired antigen.

16. The method of claim 15 wherein said non-immunogenic substrate is a polymeric material selected from carboxymethyl celluloses, monomethoxypolyethylene glycols and polyvinyl alcohols.

17. The method of claim 16 wherein said regulatory T-cells comprise $CD8^+$ cells.

18. The method of claim 15 wherein said determination of said sequence for the CDR3 region comprises determining the individual nucleotide sequences for the α- and β-chains of the T-cell receptor and effecting sequence analysis of said individual nucleotide sequences to

determine the sequences of the J-gene of both α- and β-chains.

19. The method of claim 14 wherein said desired antigen is an allergen.

20. A method of immunotherapy, which comprises administering to a host a peptide or composition as claimed in any one of claims 1 to 13.

21. The method of claim 20 wherein said host has been previously exposed to said antigen.

22. The method of claim 20 for prophylatic immunization of said host.

23. The method of claim 20 wherein said administration is effected to suppress an allergic response in said host to said antigen or to protect said host from an allergic response to said antigen.

24. The method of claim 20 wherein said administration is effected to suppress an autoimmune response in said host to said antigen or to protect said host from an autoimmune response to said antigen.

25. The method of claim 20 which is effected in conjunction with at least one administration of therapeutic molecules prone to producing unwanted immunological responses.

26. The method of any one of claims 20 to 25, in which said host is a human.

27. A method of diagnosing an allergic response of a host to an allergen, which comprises:

screening a serum from said host with a plurality of peptides as claimed in any one of claims 1 to 11 corresponding to a plurality of allergenic antigens, and

detecting reactivity of at least one of said peptides to said serum.

28. The method of claim 27 wherein said at least one peptide exhibiting reactivity is subsequently administered to the host.

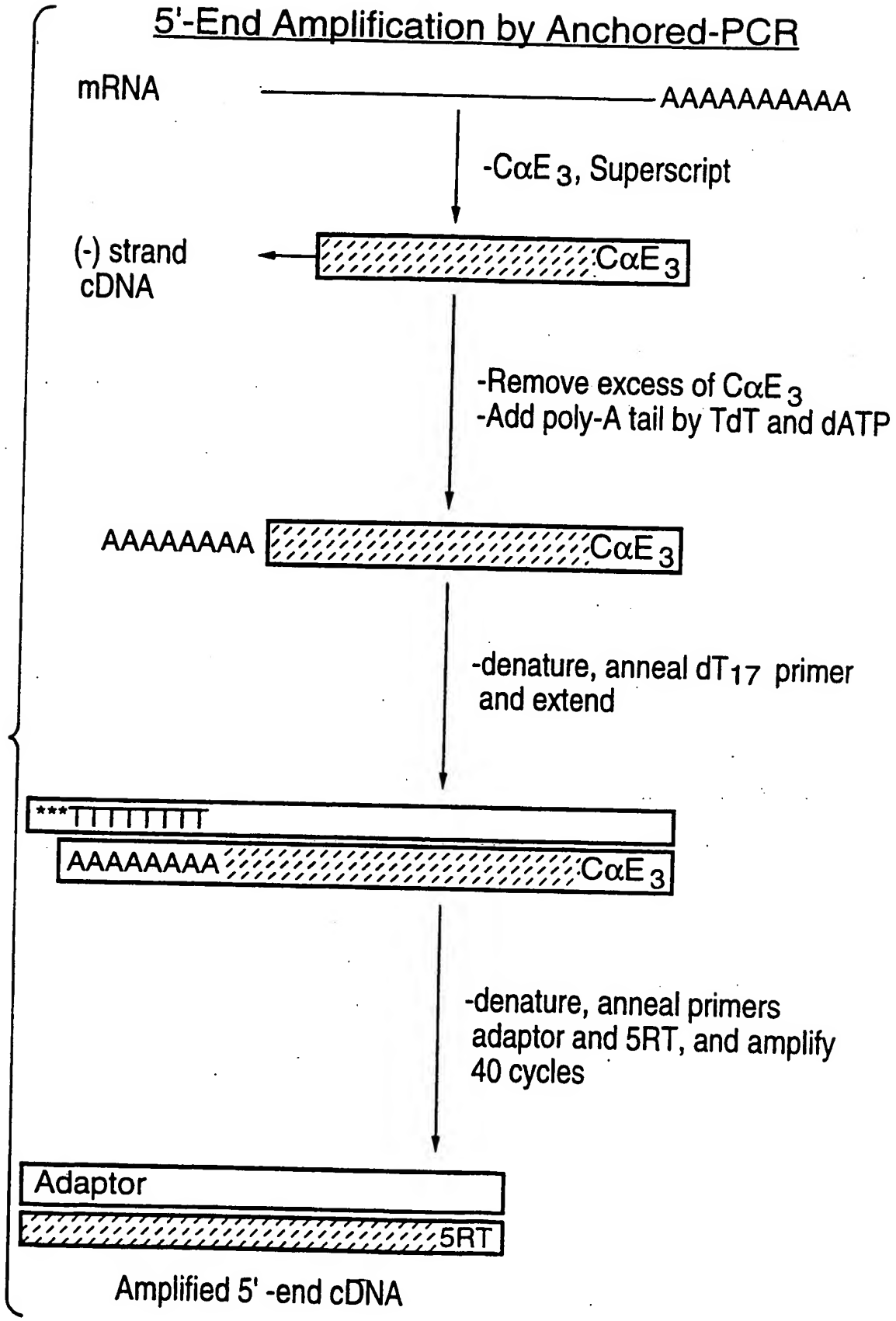


FIG.1.

SUBSTITUTE SHEET

FIG.2A.

```
TTTGCTGTCCTAGGAACCAGGTTCCACTTCAGGGTGCAGCACAGCCTTTCCTGTGACATCAATAAAGCAAGAAAA
<---------------------- 5' UNTRANSLATED ---------------------------------->

                                          -30
ATG AAC AGA TTC CTG GGA ATA TCT TTG GTG ACT CTA TGG TTT CAA GTG GCC TGG GCA
<------------------------------ LEADER PEPTIDE ---------------------------->

1                                         30                              *
AAG AGC CAA TGG GGA GAA GAG AAT CTT CAG GCT CTG AGC ATC CAG GAG GGT GAA GAT
<----------------------- VARIABLE REGION

  60                *                              90                *      *
GTC ACC ATG AAC TGC AGT TAC AAG ACT TAC ACA ACT GTT GTT CAG TGG TAC AGA CAG

      120                                             150
AAG TCA GGC AAA GGC CCT GCC CAG CTA ATC TTA ATA CGT TCA AAT GAG CGA GAG AAG

          180                                             210             *
CGC AGT GGA AGA CTC AGA GCC ACC CTT GAC ACT TCC AGC CAG AGC AGC TCC CTG TCC

               240                                  *   *       *270*
ATC ACT GGT ACT CTA GCT ACA GAC ACT GCT GTG TAC TTC TGT GCT ACT GGG GGA GGA
                           VARIABLE REGION ----------------><-------------

                   300                                             330
AGC AAT GCA AAG CTA ACC TTC GGG AAA GGC ACT AAA CTC TCT GTT AAA TCA AAC ATC
---- JOINING REGION ------------------------------------------><-----
```

FIG.2B.

```
                          360                                                 390
CAG AAC CCA GAA CCT GCT GTG TAC CAG TTA AAA GAT CCT CGG TCT CAG GAC AGC ACC
------------------ CONSTANT REGION

                              410                                                 440
CTC TGC CTG TTC ACC GAC TTT GAC TCC CAA ATC AAT GTG CCG AAA ACC ATG GAA TCT

                                  470                                                 500
GGA ACG TTC ATC ACT GAC AAA ACT GTG CTG GAC ATG AAA GCT ATG GAT TCC AAG AGC

                                      530                                                 600
AAT GGG GCC ATT GCC TGG AGC AAC CAG ACA AGC TTC ACC TGC CAA GAT ATC TTC AAA

                                          630
GAG ACC AAC GCC ACC TAC CCC AGT TCA GAC GTT CCC TGT GAT GCC ACG TTG ACT GAG

  660                                                 690
AAA AGC TTT GAA ACA GAT ATG AAC CTA AAC TTT CAA AAC CTG TCA GTT ATG GGA CTC

      720                                                 750
CGA ATC CTC CTG CTG AAA GTA GCC GGA TTT AAC CTG CTC ATG ACG CTG AGG CTG TGG
                                                  CONSTANT REGION ----------

          780
TCC AGT TGA G
------------>
```

FIG.3A.

```
                                                  *
17.A2  K S Q W G E E N L Q A L S I Q E G E D V T M N
23.A1  - - - - - - - - - - - - - - - - - - - - - - -
P14A.1                        - - V H - - - S - - V -
HVα3.1 N - - Q - - - D P - - - - - - - - - N A - - -

       *                      *     *
17.A2  C S Y K T Y T T V V Q W Y R Q K S G K G P A Q
23.A1  - - - - - - - - - - - - - - - - - - - - - - -
P14A.1 - - - T - S I - A L - - - - - - - - E - - - -
HVα3.1 - - - - - - S I N N L - - - - - - N - - R - L V H
```

FIG.3B.

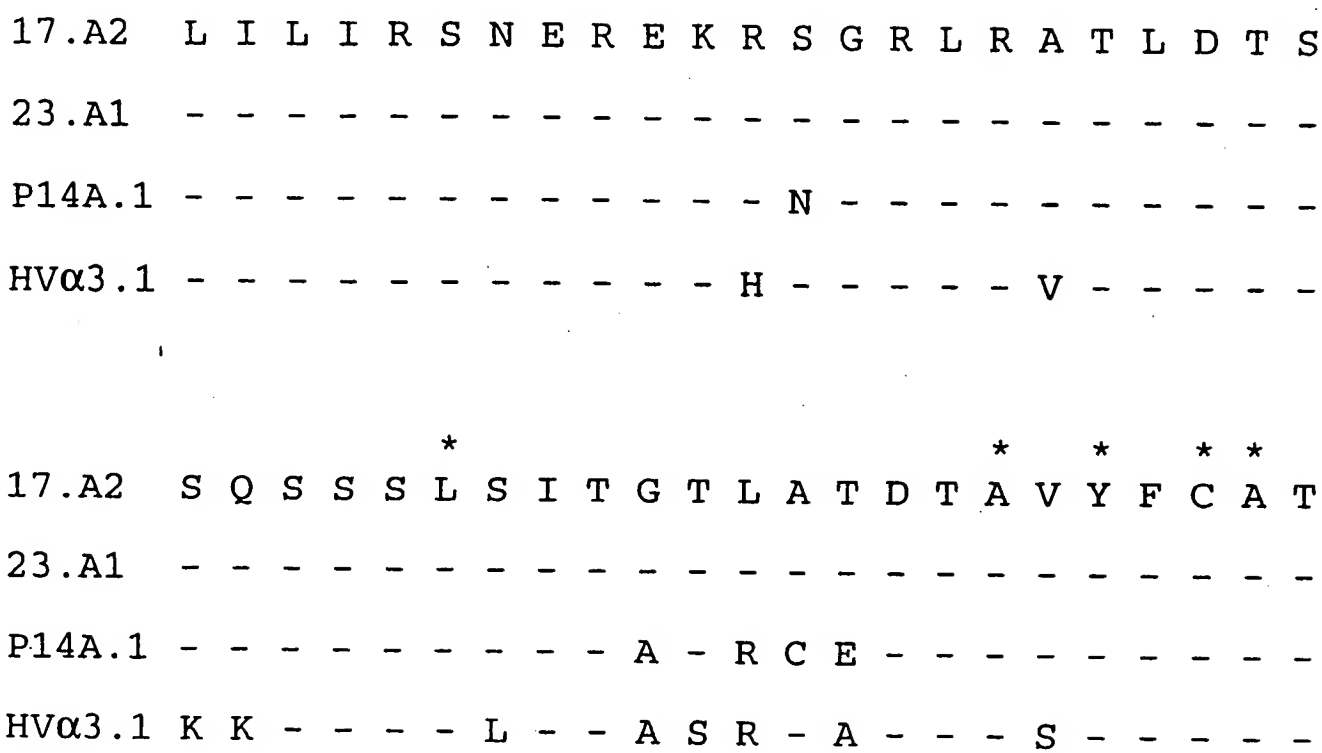
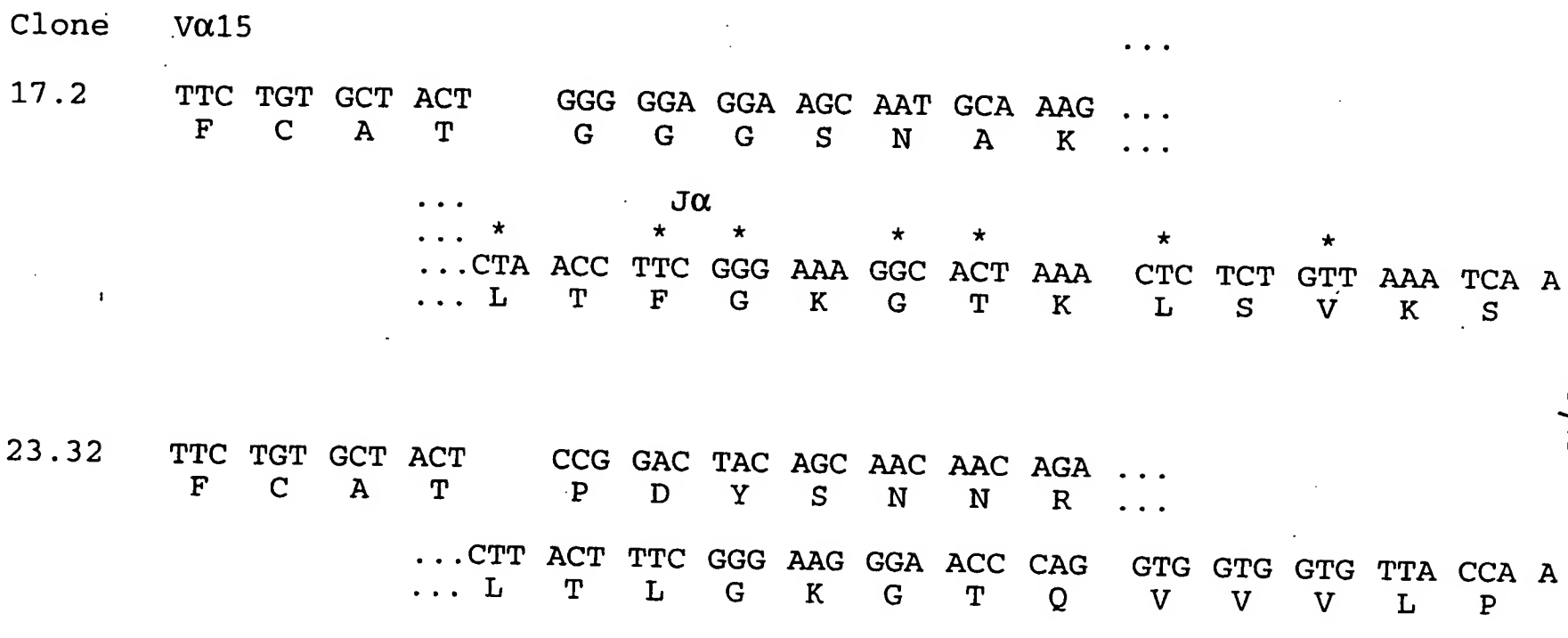

17.A2 L I L I R S N E R E K R S G R L R A T L D T S
23.A1 -
P14A.1 - - - - - - - - - - - - - - - N - - - - - - - - - -
HVα3.1 - - - - - - - - - - - - - - - H - - - - - V - - - - -
17.A2 S Q S S S L S I T G T L A T D T A V Y F C A T
23.A1 -
P14A.1 - - - - - - - - - - - A - R C E - - - - - - - - - -
HVα3.1 K K - - - - L - - A S R - A - - - S - - - - -

FIG.4.



SUBSTITUTE SHEET

FIG.5.

Clone	Vβ8.2			(N)	Dβ1.1		(N)			
17.2	TGT	GCC	AGC	GGT GAT G	CA	GGG	CCA		AAC	...
	C	A	S	G D	A	G	P		N	...
23.32	TGT	GCC	AGC	GGT GAT G	CA	GGG	TTT	AAC	CAA	...
	C	A	S	G D	A	G	F	N	Q	...

Jβ

...ACA	GAA	GTC	TTC	TTT	GGT	AAA	GGA	ACC	AGA	CTC	ACA	GTT	GTA	
... T	E	V	F	F	G	K	G	T	R	L	T	V	V	
...GAC	ACC	CAG	TAC	TTT	GGG	CCA	GGC	ACT	CGG	CTC	CTC	GTG	TTA	
... D	T	Q	Y	F	G	P	G	T	R	L	L	V	L	

7/13

SUBSTITUTE SHEET

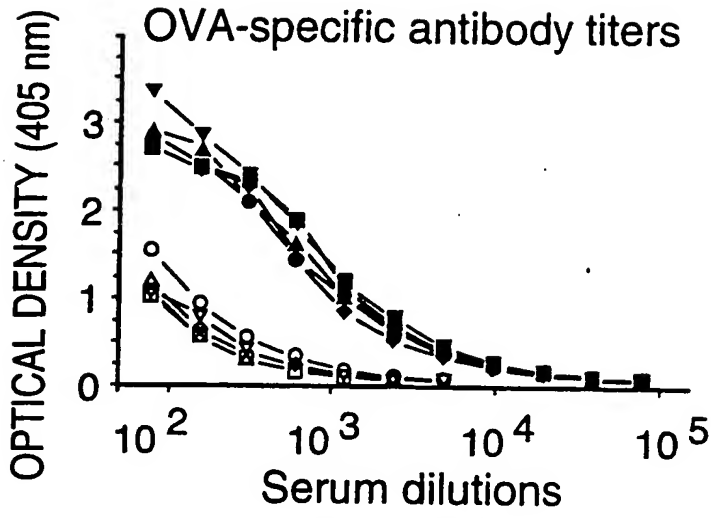


FIG.6A.

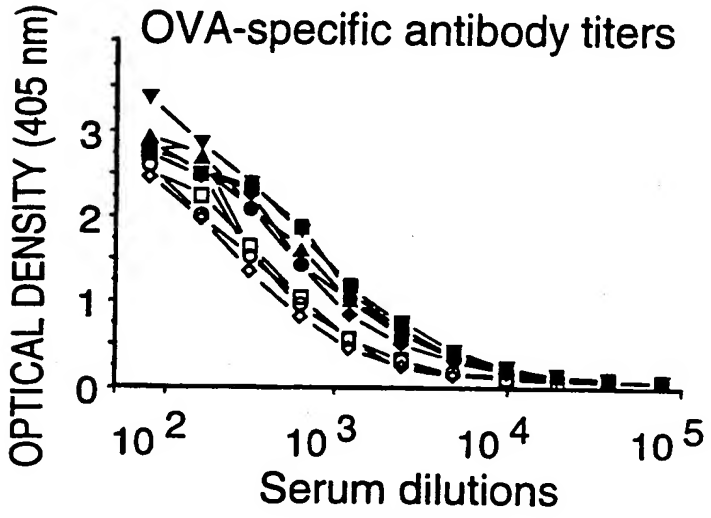


FIG.6B.

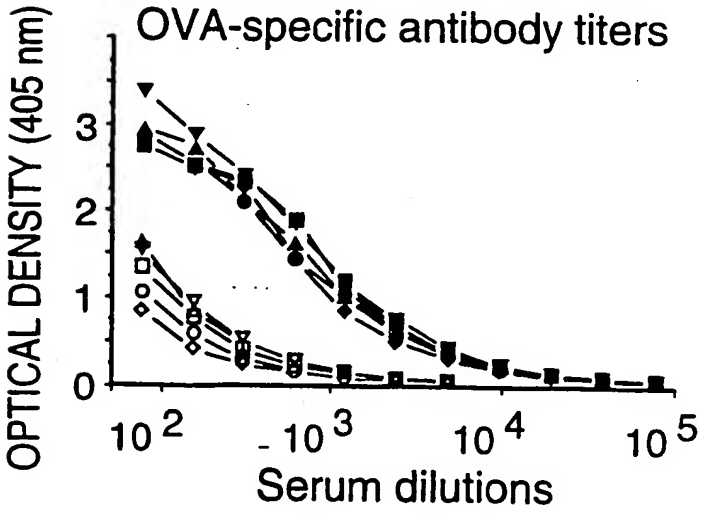


FIG.6C.

SUBSTITUTE SHEET

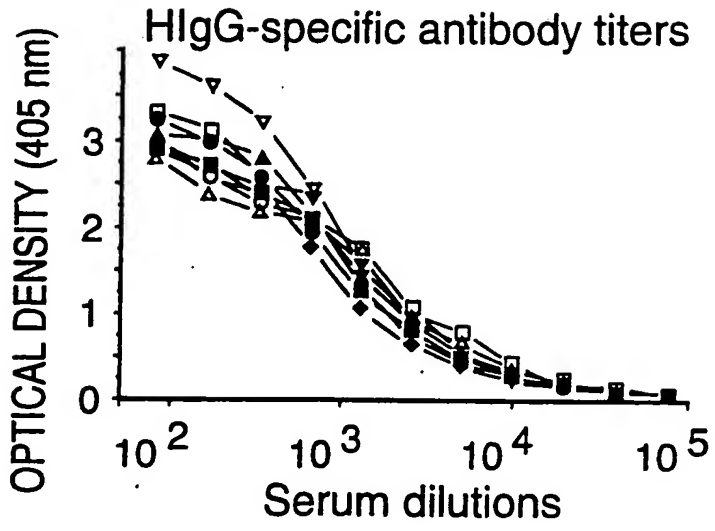


FIG.6D.

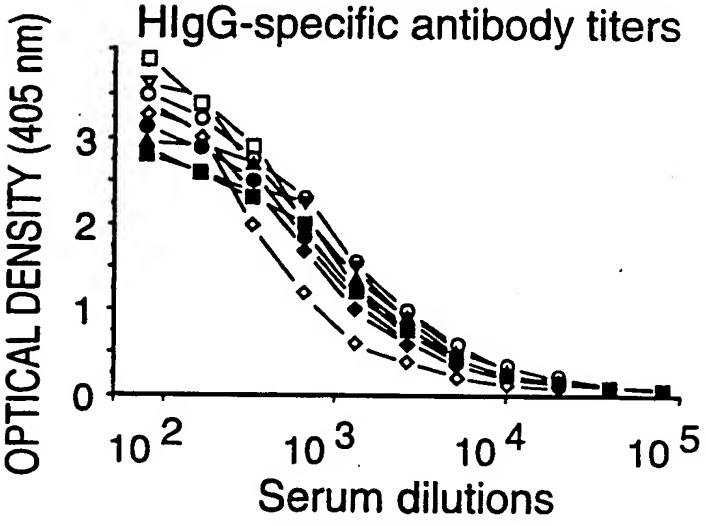


FIG.6E.

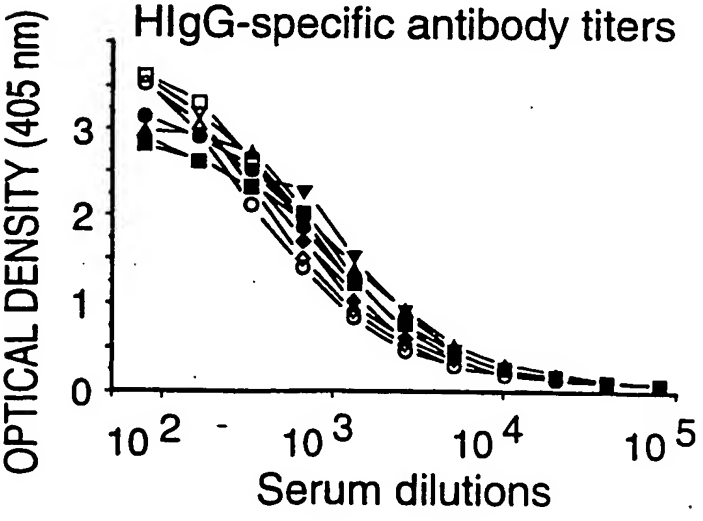


FIG.6F.

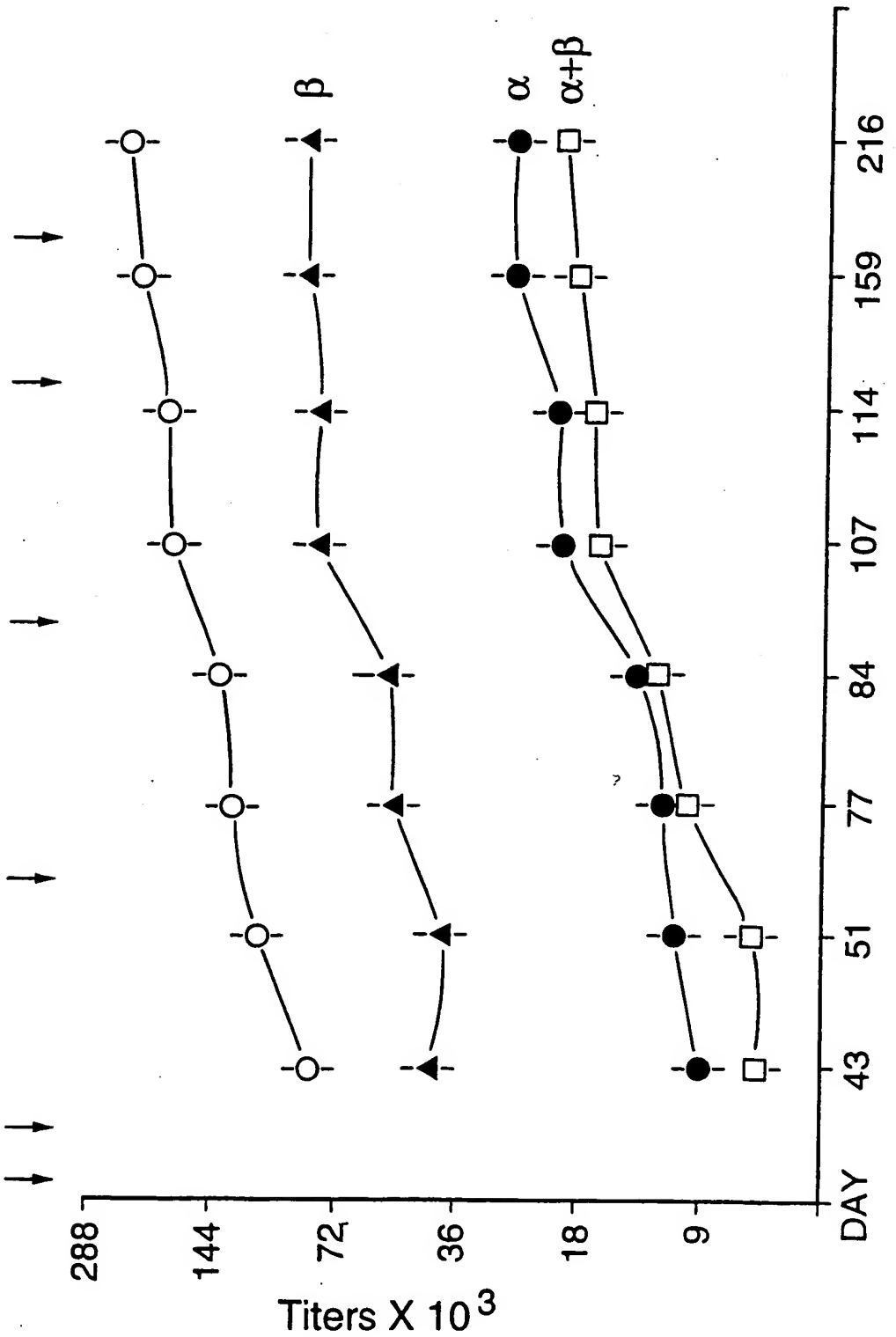

288
144
72
36
18
9
Titers X 10 3
DAY
43
51
77
84
107
114
159
216
α
α+β
β

FIG. 7.

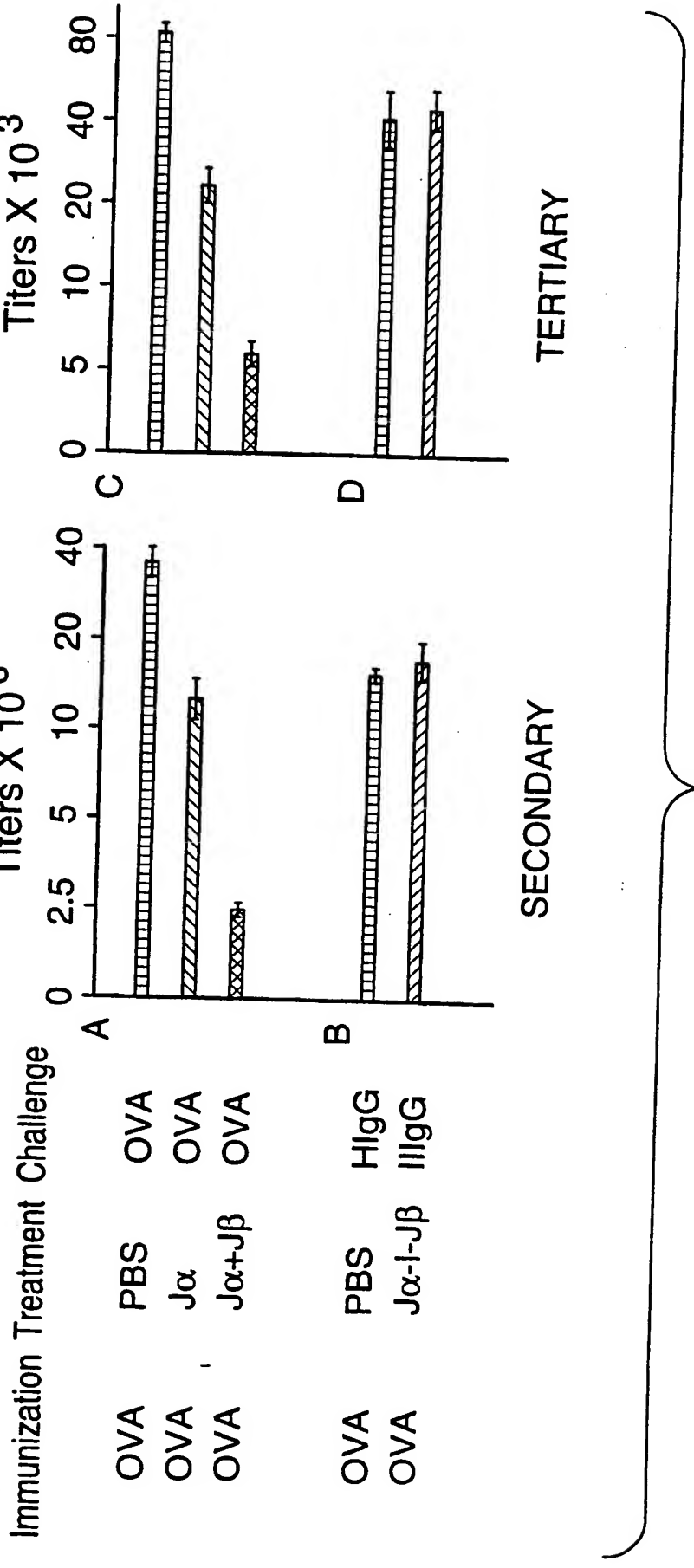

Immunization Treatment Challenge
OVA PBS OVA
OVA Jα OVA
OVA Jα+Jβ OVA
OVA PBS HIgG
OVA Jα-I-Jβ IIIgG
Titers X 10 3
0 2.5 5 10 20 40
A
B
SECONDARY
Titers X 10 3
0 5 10 20 40 80
C
D
TERTIARY

FIG.8.

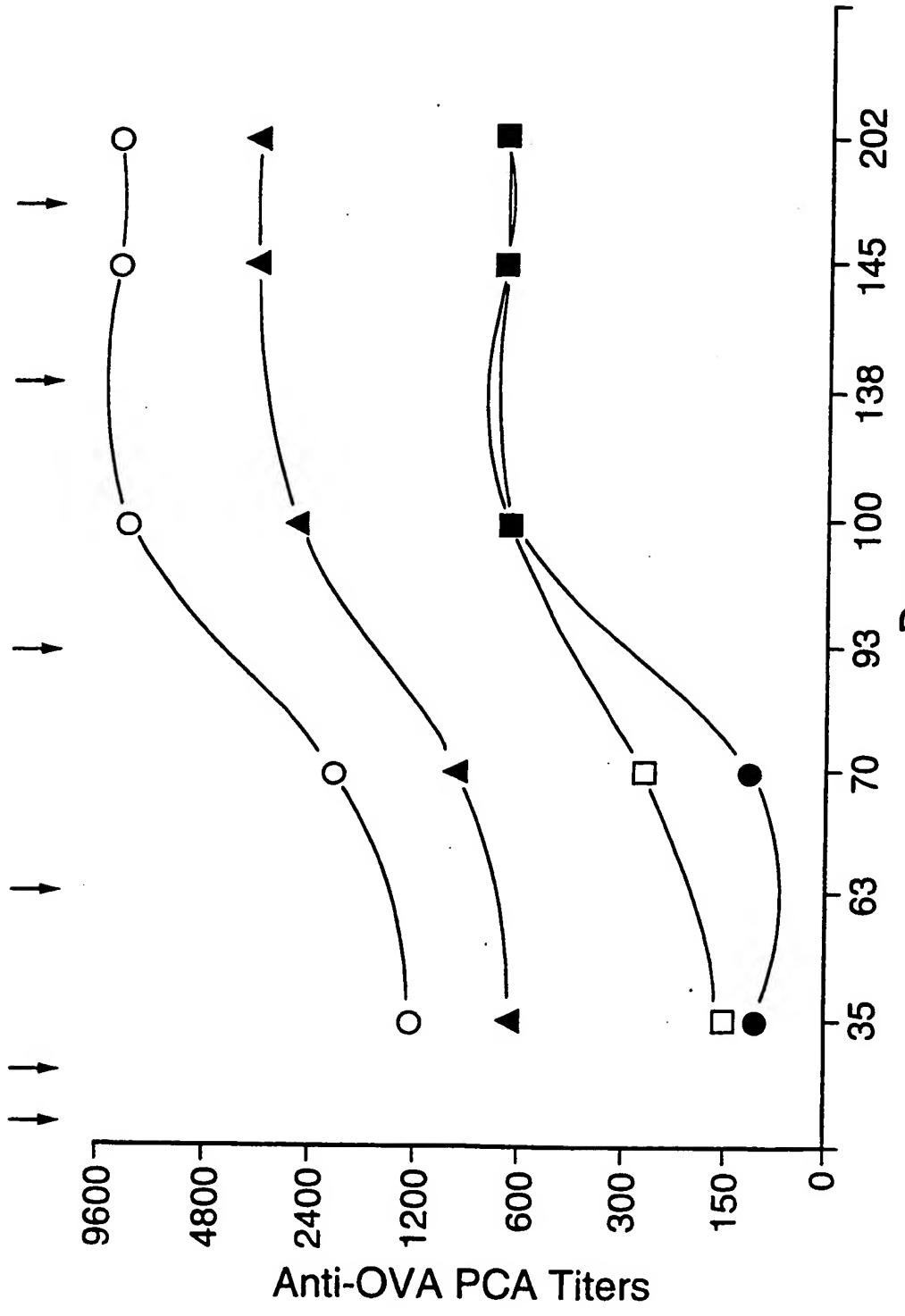

9600
4800
2400
1200
600
300
150
0
Anti-OVA PCA Titers
35
63
70
93
100
138
145
202
Days

FIG.9.

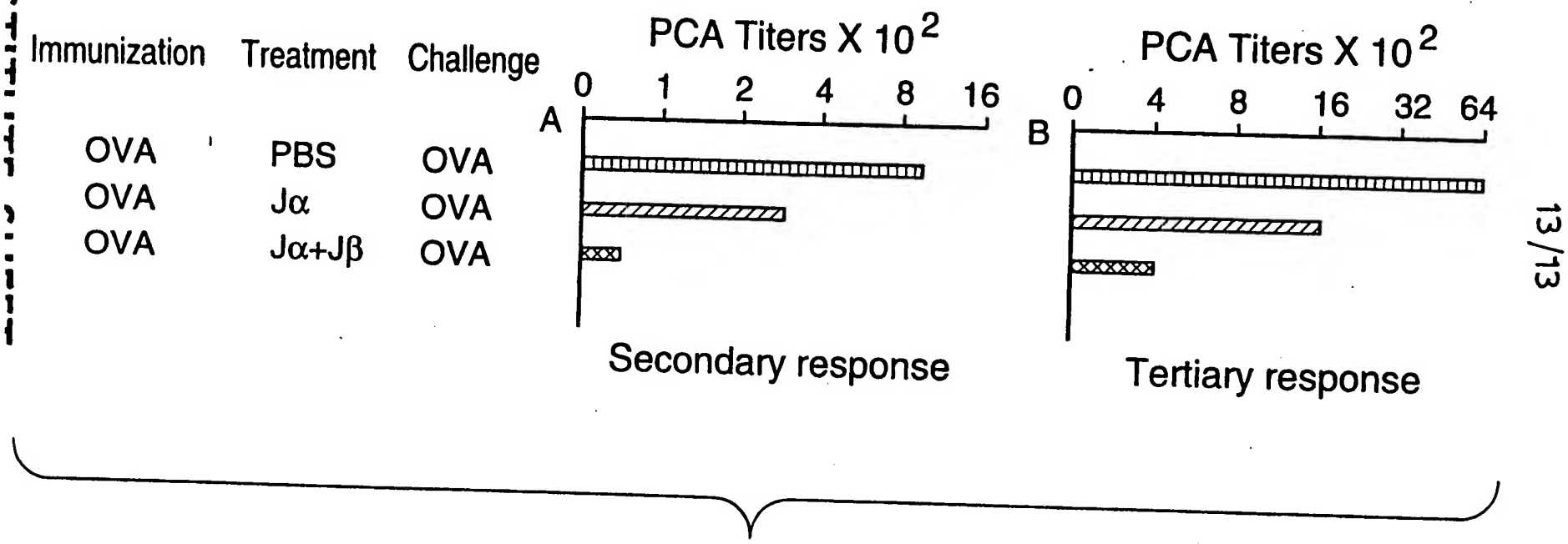

Immunization
Treatment
Challenge
OVA
PBS
OVA
OVA
Jα
OVA
OVA
Jα+Jβ
OVA
A
PCA Titers X 10 2
0
1
2
4
8
16
Secondary response
B
PCA Titers X 10 2
0
4
8
16
32
64
Tertiary response

FIG.10.

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07K15/06 A61K39/395 C07K15/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,93 04695 (THE WISTAR INSTITUTE) 18 March 1993 *see the whole document* ---	1-19,27, 28
Y	WO,A,92 21367 (VANDENBARK, A.) 10 December 1992 *page 20, lines 19-29; page 21, lines 2-4; page 35, lines 4-8; page 35, line 28-page 36, line 4; page 76, lines 11-13; page 100, lines 11-13; Table 33, page 171* ---	1-19,27, 28
Y	IMMUNOLOGY TODAY vol. 10, no. 1 , 1989 pages 10 - 14 CLAVERIE, J.-M. ET AL. 'Implcations of Fab-like structure for the T-cell receptor' *see the whole article* --- -/--	1-19,27, 28

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 August 1994	05 -09- 1994

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Marie, A

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NATURE vol. 355 , 1992 pages 224 - 230 JORGENSEN, J. L. ET AL. 'Mapping T-cell receptor peptide contacts by variants peptide immunization of single-chain transgenics' *see the whole article* ---	1-19,27, 28
Y	NATURE vol. 344 , August 1988 pages 395 - 402 DAVIS, M.M. ET AL. 'T-cell antigen receptor genes and T-cell recognition' *see the whole article* ---	1-19,27, 28
P,X	THE JOURNAL OF IMMUNOLOGY vol. 5 , 15 July 1993 pages 688 - 698 MOHAPATRA, S.S. ET AL. 'Analysis of T-cell receptor...' *see the whole article* -----	1-28

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9304695	18-03-93	CA-A-	2116526	18-03-93
		EP-A-	0602178	22-06-94
WO-A-9221367	10-12-92	AU-A-	2147292	08-01-93
		CA-A-	2110055	10-12-92
		EP-A-	0587735	23-03-94